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EFFECTS OF IONIZING RADIATIONS ON PLANT TISSUES. IV. SOME EFFECTS OF GAMMA RADIATION ON STARCH AND STARCH FRACTIONS[a,b]

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(Manuscript received July 14, 1958)

In our continued studies of the effects of ionizing radiations on plant tissues *(6, 12)*, we have dealt with various polysaccharides isolated from irradiated fruits and vegetables. In order to provide some background information for this work, model experiments were conducted on pectins and cellulose *(8, 13)*. Since starch represents a third important polysaccharide encountered in the plant tissues we are studying, a brief preliminary investigation was conducted to assess radiation effects on starch, using potato starch as an example.[c] In particular, the effects of gamma radiation were determined on solution viscosities, reducing power, and on the formation of the blue iodine complex. Amylose and amylopectin were isolated from raw starch and irradiated, and these two fractions were also isolated from irradiated starch since we were interested in determining whether the effect of gamma radiation was different in the case of amylose and amylopectin and when irradiated separately or together.

The literature on radiation effects on starch is already voluminous. However, many of these reports deal with starch irradiated with large dosages of radiations, usually in the Mrad range and reaching 400 Mrad. It is now known that under irradiation with large dosages whole series of reactions occur involving many different progressive changes in the smaller molecules formed upon the destruction of the macromolecule. In contrast to such an approach, our interest was predominantly in the incipient changes which occur with low dosages of gamma radiations which in our experiments ranged up to about 1.5 Mrad.

Although there are a number of reports in the literature dealing with the irradiation of starch, the results thus far are often inconclusive and contradictory. This is not surprising considering the lack of exact knowledge concerning the possible influence of such important factors as dose rate *(3)*, water content *(4)*, physical form of the samples, and likely many others. Khenokh *(14)* irradiated dilute solutions of potato starch with 1 g. radium

[a] Approved by the Director of the New York State Agricultural Experiment Station, Geneva, as Journal Paper 1130, June 24, 1958. This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned No. 918 in the series of papers approved for publication.

[b] Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, Ill., 1958.

[c] We are indebted to the National Starch Products, Inc. of Plainfield, N. J. for kindly providing us with a sample of raw potato starch for this work. This sample had a moisture content of 15.7%.

bromide for 20 days and observed a decrease of solution viscosity and pH and an increase is transparency and reducing power. Meanwhile the iodine complex color of the starch changed from the typical blue to a brownish color. He states that the reactions occurring were dominated by fissure of the polysaccharide linkages and oxidation of the produced fragments *(15)*. In some later work Khenokh *(16)* used a Co^{60} source and a dosage of 818,000 r for irradiating a 0.25% starch solution and found that the iodine complex absorption band shifted to a maximum at 265 mμ and that no glucose could be found in the solution. Bourne, Stacey, and Vaughan *(2)* irradiated amylose solutions with 62,500 and 125,000 rad (using a Co^{60} source) and found that glucose, maltose, maltotriose, and some other compounds were formed and that the iodine complex absorption peaks decreased and drifted to lower wavelength and that both phenomena were more pronounced in vacuum and nitrogen than in oxygen-saturated solutions.

Roberts and Proctor *(19)* found that irradiating potato segments in an ice-water bath with a total dosage of 3 Mr cathode rays did not change the physical appearance of starch grains. In a later publication *(18)* Roberts states that not until a dose of above 10 Mr do appreciable changes take place in the starch grain. The threshold value of irradiation effect appeared to be around 5 Mr and traces of fructose, glucose, and dextrin were detected at this dosage. This author concludes that "in the cold sterilization of foods containing starch, the "starch" content may be expected to remain physically and chemically unchanged up to an irradiation dose of $4\text{-}5 \times 10^6$ rep." This is in harmony with the observations of Wolfrom and associates *(24, 25)* that dry, powdered corn and potato starch, irradiated at the temperature of ethanol-dry ice baths with cathode rays up to 2 Mrep produced no discernible changes in gross appearance and behavior. Irradiation with 5 Mrep and above modified the starches to take on some of the characteristics of dextrins of the British gum type. Waxy maize starch (which is constituted almost entirely of amylopectin) when irradiated under similar conditions with 400 Mrep, turned yellow and water-soluble and was found to contain D-glucose and other degradation products.

METHODS AND MATERIALS

Irradiation. Irradiation with gamma rays was performed in the same manner as described in our previous reports in this series (6) [4]. Briefly, the dry samples were irradiated in small glass screw-top vials placed on shelves fixed at defined distances from the Co^{60} source. Dosage was governed mostly by the distances from the source and the length of irradiation, which was usually overnight. The dosimetry used has been noted in a previous publication *(6)*. After irradiation the samples were brought to Geneva and stored in a closed chamber until used. The samples contained 15.7% moisture and therefore were supposed to be free from radiation after-effects (7).

Starch solutions. A survey of the literature indicates that drastic procedures have often been applied by investigators for the dissolution of starch, including autoclaving *(20, 21)*, heating with strong alkali *(9, 17)*, and others. Ethylene diamine has been also used for this purpose *(1)* but like Foster and Hixon *(5)*, we have encountered difficulties in obtaining satisfactory solutions. A procedure was desired where the chances of excessive degradation during dissolution were minimized. Grinding the starch in a small mortar

[4] We wish to express our sincere appreciation to Dr. L. W. Tuttle and the Atomic Energy Project, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y. for their continued cooperation.

with a small amount of Berkshire sand with successive small volumes of 1 *N* KOH followed by centrifuging gave a satisfactory solution providing the grinding was repeated at least 3 times and 1 *N* KOH was used for washing the sample back into the mortar and for making up to the final volume. We had no difficulty in preparing 0.4% solutions in this manner. Subsequent work showed that the same dissolution can be accomplished in a simpler manner by successive grindings without the sand in a Potter-Elvehjem Pyrex tissue grinder. As expected, irradiated starch was always easier to dissolve than the original raw starch. No grinding was required for dissolving amylose and amylopectin in the 1 *N* KOH.

Separation of amylose and amylopectin. The method described by Schoch *(22)* for the separation of the 2 starch components was used for this purpose. Briefly, the method was as follows: Solutions representing 2 g. of starch made up as described above were centrifuged, transferred to a 600 ml. beaker, and the pH of the solution was adjusted to between 5.9 and 6.3. Now enough n-butanol was added dropwise under vigorous stirring to give a final concentration of 80% in the mixture which was then stirred for 2 additional hours and allowed to stand at room temperature overnight. Next morning the precipitated amylose was centrifuged off and washed with 50 ml. 8% butanol in water, centrifuged, and placed in 95% ethanol. The ethanol was changed several times to accomplish dehydration and was finally changed to absolute ethanol. Afterwards the amylose was transferred to a weighing dish and dried in vacuum over P_2O_5. The mother liquor and butanol washings from the above procedure were collected and ¼ of the volume of 95% ethanol was added under vigorous mechanical stirring. The mixture was kept at 4° C. overnight, the clear liquid siphoned off, the precipitated amylopectin was separated by centrifuging and then dehydrated and dried as described above. A large number of such separations were made for practice and then 6 fractions were combined for the accumulation of amylose and amylopectin for irradiation.

The proportion of amylose and amylopectin in the 6 (unirradiated) preparations combined for the latter use averaged 20.9% amylose and 79.1% amylopectin. Amylose and amylopectin were also isolated from starch irradiated with various dosages of gamma rays. Here the amylose yields averaged 21.1% and the amylopectin yields 76.3% whereas the average total recovery of the 2 fractions from starch was 97.3%.

Viscosity determinations. Solution viscosities were determined in 0.4% solutions after additional centrifuging. Ostwald-Cannon-Fenske viscometers were used at 25° C. and the viscosity value of the 1 *N* KOH was used in the calculation of the relative and specific viscosities (relative − 1). Additional viscosity determinations were made in 3 dilutions (with 1 *N KOH*) and the intrinsic viscosity values were calculated in the usual manner *(8)* by plotting specific viscosity / C as function of the concentration, C, and extrapolating the curves to zero concentration. Inasmuch as the intrinsic viscosity values moved parallel with the specific viscosity values, only a few of the former are shown in Table 1.

Measurement of iodine-complex color. The iodine complex color was measured in the usual manner *(1, 10, 17)* in dilute solutions adjusted to about pH 4.5 and after the addition of sufficient quantity of a solution containing 0.2% iodine and 0.2% potassium iodide to give a final iodine concentration of 0.002%. The optical densities were read at various wavelengths with a Model B Beckman Spectrophotometer. This procedure was tested for the effects of the proportion of iodine added, for the effect of small pH differences and other factors and was found satisfactory. The results obtained on the various samples are shown in Figure 2.

Determination of reducing power (free reducing groups). Several methods were tried for the determination of the reducing power of our samples. The copper reduction method of Bertrand *(11)* gave unreliable results on account of the apparent precipitation of starch by the copper solution. The hypoiodite method gave unreliable results on account of the presence of the large proportion of starch (or starch fraction). The method found most suitable was that of Sumner *(23)* applying dinitrosalicylic acid reagent. For the determinations the reagent was prepared as described by Sumner and his procedure was followed throughout except that the optical densities of the finally diluted reaction mixtures were measured at 490 mμ using a Model B Beckman Spectrophotometer. Difficulties experienced in dissolving some samples were overcome by allowing the sample to dissolve in alkali. Glucose solution was used for establishing the calibration curve which showed, for the region used, a straight-line relationship between optical density

and glucose concentration. The reducing power values were expressed in terms of anhydroglucose. The reliability of the method was carefully tested. For instance, with an irradiated starch sample, using 90 mg. samples reducing power values of 0.35 and 0.36 were obtained, while using 130 mg. samples 0.35 and 0.36 were obtained. Similar testing with other preparations gave correspondingly satisfactory results.

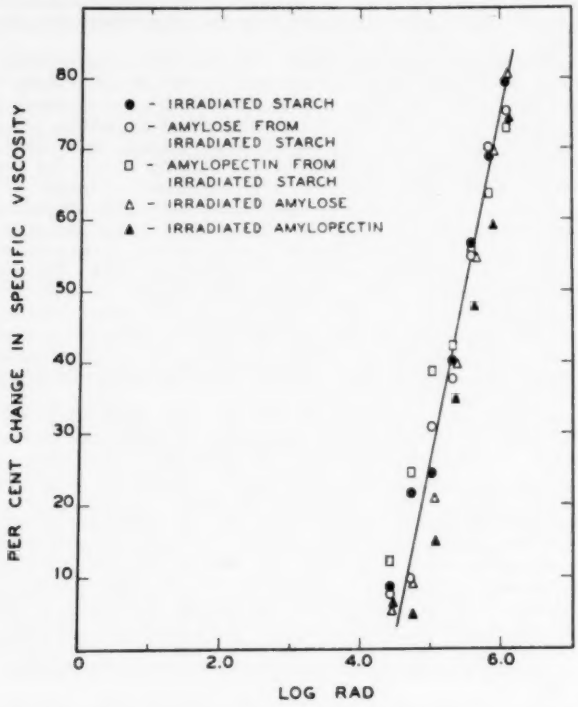

Figure 1. Effect of gamma irradiation of dry starch and starch fractions on solution viscosities in 1 *N* KOH.

RESULTS AND DISCUSSION

The results of viscosity determinations on the various samples are shown in Table 1. Considering the fact that all the experiments reported in this table represent single runs and not averages, the agreement in the viscosity values is quite satisfactory. A good relationship exists between the radiation-induced viscosity changes in starch on one hand, and amylose and amylopectin on the other. Similarly, the agreement between the viscosity values of the various irradiated amylose and amylopectin samples and the results of viscosity determinations on these 2 components isolated from irradiated starch all seem to follow the same pattern. This fact is illustrated in Figure 1 in which the changes in the specific viscosities are plotted for the 5 groups of samples and are represented by a single line. Even if some

TABLE 1

Observations on irradiated starch, amylose and amylopectin

Dosage, rad x 10^{-3}	Irradiated starch			Amylose from irradiated starch			Amylopectin from irradiated starch			Irradiated amylose			Irradiated amylopectin		
	Intrinsic visc.	Spec. visc.[1]	RP [2]	Yield %	Spec. visc.	RP	Yield %	Spec. visc.	RP	Intrinsic visc.	Spec. visc.	RP	Intrinsic visc.	Spec. visc.	RP
0	1.92	1.71	0.16	21.1	1.55	0.21	81.0	1.30	0.13	2.09	1.43	0.21	1.61	1.18	0.14
23	1.80	1.56	0.21	18.9	1.43	0.26	78.9	1.14	0.12	1.84	1.35	0.23	1.55	1.11	——
44	1.79	1.34	0.22	18.9	1.40	0.22	76.9	0.96	0.12	2.10	1.30	0.22	1.63	1.13	0.16
91	1.47	1.29	0.28	22.0	1.07	0.24	52.0	0.80	0.22	1.88	1.13	0.34	1.42	1.01	0.19
183	1.29	1.02	0.36	23.0	0.97	0.30	81.6	0.75	0.14	1.36	0.86	0.38	1.22	0.77	0.31
366	0.95	0.74	0.52	23.2	0.70	0.38	79.0	0.58	0.18	0.92	0.64	0.47	0.79	0.62	0.36
731	0.79	0.54	0.96	21.8	0.46	0.61	81.6	0.48	0.20	0.66	0.44	0.77	0.68	0.49	——
1462	0.47	0.35	1.47	19.8	0.39	0.78	78.9	0.36	0.40	0.35	0.28	1.38	0.42	0.31	——

[1] Specific viscosity of 0.4% solution in *N* KOH at 25° C.

[2] Reducing power expressed as percent anhydroglucose.

groups of points would fit better a parallel line somewhat to the right or left from the one shown in the figure, this deviation could be only small. The line representing all data crosses the abscissa at 60,000 rad; thus this might be taken as the approximate threshold dosage of the radiation degradation for the 5 sets of samples depicted in Figure 1 as measured by viscosity changes. One might conclude that amylose and amylopectin are degraded by gamma radiations at a similar rate and that this degradation is similar whether the 2 fractions are irradiated together in their natural association in starch, or separately. Further, it is quite obvious from these data that the radiation-induced degradation in these samples commences at some dosage around 60,000 rad and then follows the straight-line pattern of change vs. log dosage described previously *(8, 13)*.

As one would expect, the intrinsic viscosity values run parallel to the specific viscosity values. The viscosities of the unirradiated amylose sample and that of amylose isolated from unirradiated starch are somewhat higher than the coresponding values for amylopectin.

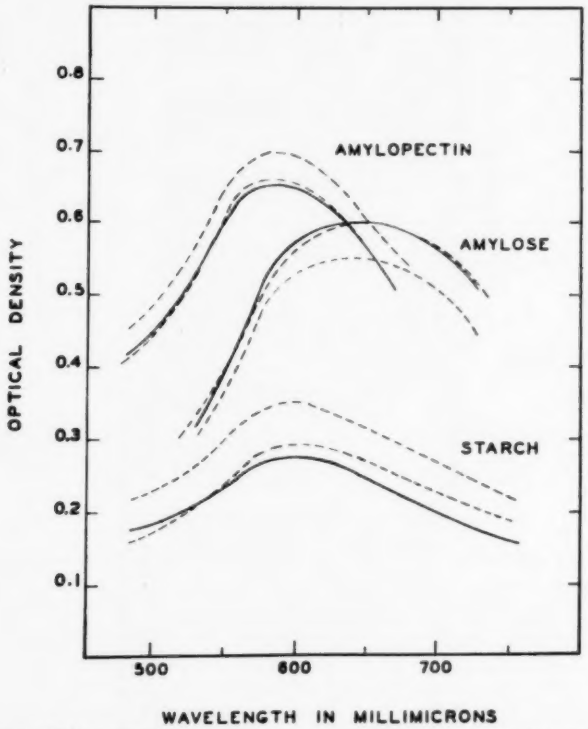

Figure 2. Iodine-complex absorption curves of starch (0.01%) and amylose and amylopectin (0.04%) irradiated with gamma rays. Solid lines indicate absorption curves of unirradiated control samples, dotted lines show lower and upper limits of measurements on 7 samples irradiated with dosages up to 1.5 Mrad.

The reducing power (RP) values, also given in Table 1, are more difficult to interpret than the results of viscosity determinations. The unirradiated raw potato starch, which served as starting material for all these tests, showed a RP value of 0.16, indicating a degree of polymerization (DP) of 625 or an average molecular weight around 100,000. The 2 samples of unirradiated amylose (which were derived from different batches) had identical RP values of 0.21 indicating a DP of 476. The 2 samples of amylopectin gave RP values of 0.13 and 0.14, corresponding to an average DP of 740. It seems fruitless to cite and discuss similar information on the RP of starch and starch fractions from the literature since the methods of fraction preparation and DP estimation have a well-appreciated profound effect on the results obtained. In addition to the natural variations, the drastic methods used in preparing starch solutions are likely responsible for the wide variety of values found in the literature.

From the standpoint of the present study, the important point is that the RP values changed slowly and somewhat indefinitely up to a dosage of 91,000 rad. It must be borne in mind that viscosity is a weight-average molecular weight property whereas RP indicates changes in the number-average molecular weights *(11)*. Therefore it is not surprising that the viscosity changes are more pronounced than the RP changes in the samples exposed to low dosages of radiations. The RP values in the irradiated amylose and amylopectin seemed to increase somewhat faster than in the amylose and amylopectin isolated from the irradiated starch. On the other hand, the viscosity values decreased at approximately the same rate in all samples and therefore these differences in the RP values would have to be confirmed before much weight can be attached to them. This matter of determining the RP in polysaccharides of large molecular weights is a well recognized problem *(11)* and the interpretation of the results of RP determinations is still under debate.

The measurements of the color of the iodine complex (Figure 2) led to some interesting observations. Under our conditions potato starch showed an absorption peak at 600 mμ, near the 628 mμ found by Baldwin, Bear, and Rundle *(1)*. The absorption peaks for amylose and amylopectin were near 630 and 580 mμ, respectively. Upon irradiation of starch, amylose and amylopectin with up to 1.5 Mrad gamma rays, no definite progressive changes in the absorption peak or in the extent of absorption could be observed. There is a slight but inconsistent variation in the absorption curves for the various degrees of irradiation, indicating no definite trends. These variations might be the results of the noticeable changes which occur in the solubility of starch and starch fractions when they are irradiated. Solutions from irradiated materials seemed to be clearer than the solutions made from unirradiated starch, amylose, and amylopectin. Thus the major conclusion from our measurements of the iodine-complex formation is that under the conditions used this property of starch, amylose, and of amylopectin is not changed by gamma radiation up to 1.5 Mrad. Our results seem to be in disagreement with those of Khenokh *(16)* who found a drift in the absorption peak of the iodine complex of irradiated starch solutions at gamma radiation dosages covered by the range used in the present work. Bourne, Stacey, and Vaughan *(2)* also observed such a drift in the absorption peaks of

amylose solutions. The explanation of this apparent discrepancy might lie in the fact that starch (and starch fractions) may be more susceptible to radiations when in solution than when in the dry form. Such a behavior is easily understandable on the basis of present concepts of radiation effects. A significant difference in the radiation susceptibility of pectin and pectin solutions has been reported from this laboratory *(13)*. Ehrenberg, Jaarma, and Zimmer's report *(4)* on the role of water on the effect of ionizing radiations on starch does not provide a clear picture of this situation and therefore further reports on this topic must be awaited before these differences can be explained with certainty.

The point of starch degradation where no iodine-complex formation occurs has been designated as the "achroic point" and the RP where this occurs is the "achroic-R-value." Clearly, the achroic point is not even approached in our experiments in spite of the fact that the radiation effect was sufficient to destroy the typical viscosity of starch and starch fractions.

SUMMARY

Raw dry potato starch and amylose and amylopectin isolated from it were irradiated with gamma rays up to 1.5 Mrad. Amylose and amylopectin were also isolated from irradiated starch. Solution viscosities in 1 *N* KOH, the proportion of free reducing groups, and the iodine-complex absorption curves were determined for a series of seven irradiation dosages.

The viscosity changes followed a similar pattern in all five groups of measurements. The proportion of reducing groups increased less distinctly at low dosages but definitely above 100,000 rad. No definite changes could be observed in either the wavelength position of the absorption peaks of the iodine complex curves or in the extent of absorption of the iodine-complex of the irradiated samples as compared with the unirradiated controls. The results indicate that amylose and amylopectin are degraded by gamma radiation in the same manner whether irradiated together in the form of starch or as separate fractions.

Using dosages up to 1.5 Mrad, the solution viscosities of starch, amylose, and amylopectin could be greatly reduced by gamma radiation without simultaneously affecting the iodine-complex absorption curves of these polysaccharides.

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EFFECTS OF CATHODE RAY AND GAMMA RAY IRRADIATION ON SOME ORGANIC ACID-CARBOHYDRATE SYSTEMS[a, b]

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Use of ionizing radiation for the cold sterilization of heat sensitive foods has been the subject of intensive investigation in recent years. The thermal processing and storage of many products results in darkening or "browning" due to various types of nonenzymatic degradative reactions. In many instances, the Maillard reaction — the interaction of amino acids and reducing sugars — is involved, but in the case of some fruit products at least, organic acid-reducing reactions appear to take place. Since some of the products susceptible to this type of browning, such as juices, jellies and apple products have been studied in relation to radiation sterilization *(2, 11, 12, 13, 14, 15)*, it was deemed desirable to study the effects of irradiation upon the browning precursors and the browning of several organic acid-reducing systems. Sugar solutions, sugar-acid solutions, pectin gels and apple sauce were selected because of the availability of previous data on the effects of thermal treatments upon the composition of these products *(7, 8, 9, 10, 16)*.

It was the purpose of this investigation to determine the nature and severity of physical and chemical changes incurred by the above-mentioned organic acid carbohydrate systems under exposure to various dosages of cathode and gamma ray irradiation, to compare the effects with those of heat treatment, and to determine the effect of pre-irradiation and post-irradiation heating on these systems.

EXPERIMENTAL

Preparation of sugar and sugar acid solutions. One series of solutions containing 60% sucrose, 30% fructose, and 30% glucose was made up using distilled water. Another series of solutions containing 60% sucrose, 30% fructose and 30% glucose was made up using a 1% malic acid solution as diluent. A third series of solutions containing 12% sucrose, 6% fructose and 6% glucose was made up using an 0.5% malic acid solution as diluent. The fourth series consisted of solutions of 60% sucrose, 30% fructose and 30% glucose using an 0.5% citric acid solution as diluent. All reagents used were of C.P., A.C.S. or reagent grade.

Two ml. of each of the solutions were filled into 10 ml. Kimble Neutraglass ampules[e] for cathode ray irradiation, while 10 ml. portions were filled into 18 × 150 mm. Pyrex test tubes for gamma ray irradiation. The ampules and tubes were sealed in an oxygen

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[e] Manufactured by the Kimble Glass Co., Toledo 1, Ohio.

flame. In addition, one-half pint canning jars with metal covers, were filled with each of the solutions.

Preparation of pectin gel. The composition of the gels was identical to that reported by Livingston *et al. (10)*. The method of preparation was as described by Steinberg *et al. (16)*.

Three ml. portions of hot gel were filled into Pyrex tubes 9mm. O. D.; 1 mm. wall thickness). Ten ml. portions were filled into 18 × 150 mm. Pyrex test tubes. Petri dishes containing 20 ml. portions of gel, one-half pint canning jars containing about 235 ml. were also used.

Preparation of apple sauce. Fresh McIntosh apples were used in preparing apple sauce using the method described by Livingston *et al. (9)*. Sweetened apple sauce contained 3 ounces of added refined sugar per pound of prepared fruit. Some of the unsweetened apple sauce was fractionated into 2 parts, i.e. filtrate and pomace, by filtering it through four-fold thicknesses of cheesecloth. The apple sauce pomace was subsequently washed 15 times with cold water to remove most of the soluble constituents. The resulting "pomace" was low in solubles but was not pressed and retained therefore considerable moisture.

Apple sauce and apple sauce pomace were filled into one-half pint canning jars, while the apple sauce filtrate was filled in 10 ml. portions into 18 × 150 mm. Pyrex tubes. Composition of the 3 fractions is shown in Table 1. Analysis were performed in accordance with *Methods of Analysis* of the A.O.A.C. *(1)*.

Irradiation of samples. All samples were prepared within 24 hours prior to shipment to the site of irradiation, and post-irradiation determinations were carried as soon as possible after irradiation. Samples subjected to cathode ray irradiation [f] using a 2 MeV Van de Graaff accelerator, were packed in ampules, 9 mm. tubes or Petri dishes. Samples irradiated by gamma rays [g] were packed in 18 × 150 mm. tubes or half-pint jars enclosed in No. 2 cans. Irradiation dosages requested were 0.465, 0.93, 1.86, 3.72, 7.44 and 14.88 megarads. All samples were shipped and irradiated at ambient temperature, except for the apple products which were shipped to and from the irradiation site in the frozen state, but were thawed prior to irradiation.

TABLE 1

Composition of apple sauce products.

Product	Total Sugars	Acid (as malic)	Moisture
	%	%	%
Apple sauce	11.8	0.34	87.5
Apple sauce pomace	0.9	0.01	96.6
Apple sauce filtrate	13.2	0.34	86.2
Sweetened apple sauce	19.2	0.34	79.2

Analytical measurements. As an index of discoloration, the optical density or the reflectance at 450 mμ was determined using a Beckman Model DU Spectrophotometer [h]. Ultraviolet absorption curves were also obtained with this instrument. Dilutions were made wherever necessary. The pH of the samples was measured with a Beckman Model G pH meter. Total acidity was determined by electrometric titration using the same meter. Samples were checked for fluorescence using a Model SL 3660 ultraviolet lamp equipped with a long wave filter [i]. Viscosity was measured at 40° F. ± 2° F. (3.5° − 5.5° C.) in a 100 ml. beaker using a Brookfield viscometer [j] equipped with a No. 5 spindle which was operated at 20 R.P.M. and was allowed to rotate for one minute before the reading was recorded.

Heating of samples. All samples of solutions and gels were heated, wherever so indicated, to 230° F. (110° C.) for 30 minutes in an oil bath equipped with a deKhotinsky thermoregulator. At the end of the heating period, the tubes were rapidly cooled in an ice

[f] At the High Voltage Engineering Corporation, Cambridge, Mass.
[g] At the Argonne National Laboratory, Lemont, Ill.
[h] Manufactured by Beckman Instruments Inc., Fullerton, Calif.
[i] Manufactured by Ultraviolet Products Inc., San Gabriel, Calif.
[j] Manufactured by the Brookfield Engineering Laboratories, Stoughton, Mass.

water bath. Samples of all apple products were heated in a water bath at 212° F. (100° C.) for 30 minutes and air-cooled in a refrigerator.

Preparation of 5-hydroxymethylfurfuraldehyde solutions. The method of Haworth and Jones *(5)* was used in synthesizing 5-hydroxymethylfurfuraldehyde (HMF). Aqueous solutions containing 0.1% HMF were prepared using distilled water. Two ml. portions of solution were filled into 10 ml. ampules, the headspace air was replaced by nitrogen, and the ampules were sealed in an oxygen flame.

RESULTS AND DISCUSSION

Typical data obtained in these experiments are presented in Tables 2-9 and Figures 1-4. For the sake of brevity, much additional information obtained on the effects of dosages intermediate to those shown, or the compar-

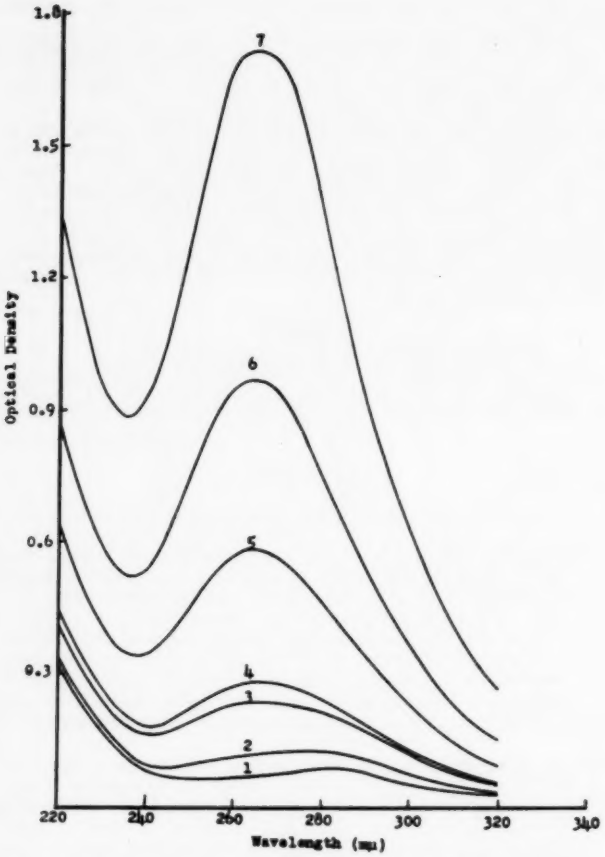

Figure 1. Effect of gamma ray irradiation on the ultraviolet absorption spectrum of unheated pectins gels.

1 — unirradiated; 2 — 0.465 megarads; 3 — 0.93 megarads; 4 — 1.86 megarads; 5 — 3.72 megarads; 6 — 7.44 megarads; 7 — 14.88 megarads.

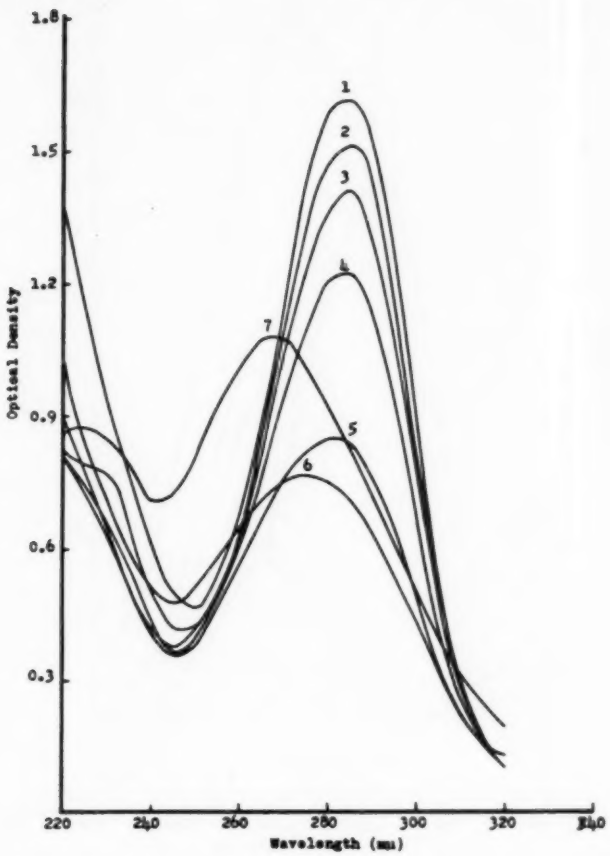

Figure 2. Effect of gamma ray irradiation on the ultraviolet absorption spectrum of pectin gels heated at 230° F. for 30 minutes prior to irradiation.

1 — unirradiated; 2 — 0.465 megarads; 3 — 0.93 megarads; 4 — 1.86 megarads; 5 — 3.72 megarads; 6 — 7.44 megarads; 7 — 14.88 megarads.

ative effects of dosages intermediate to those shown, or the comparative effects of gamma and cathode ray irradiation, and on ultraviolet absorption has been omitted.

Tables 2, 3 and 4 illustrate the effects of the 2 types of radiation upon darkening, acidity and ultraviolet absorption of the sugar solutions. Tables 5 and 6 relate to the effects upon the sugar-acid solutions; Tables 7 and 8 summarize the changes in pectin gels and apple sauce, respectively; Table 9 shows the effect of irradiation on 5-hydroxymethylfurfuraldehyde solution.

Effect of irradiation on sugar and sugar-acid solutions. Both gamma ray and cathode ray irradiation produced a yellow discoloration in the sugar

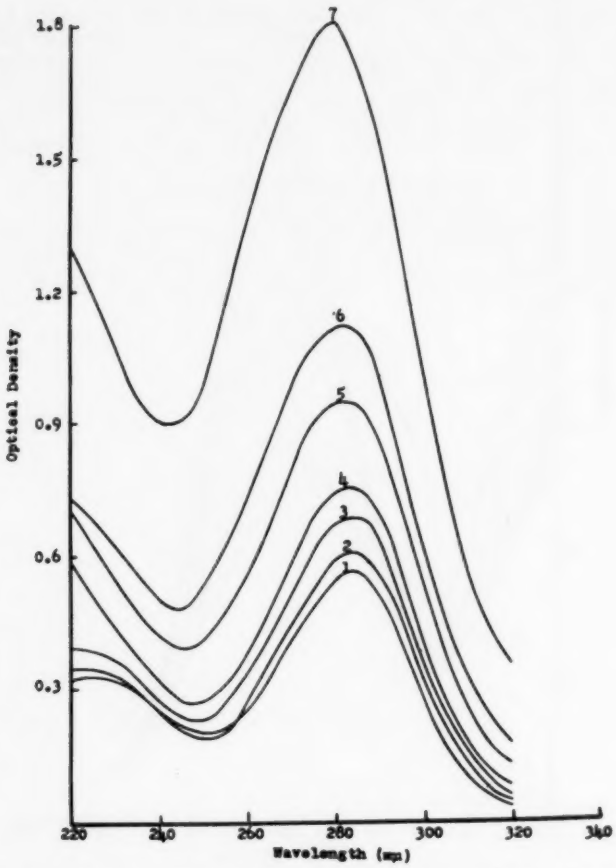

Figure 3. Effect of gamma ray irradiation on the ultraviolet absorption spectrum of pectin gels heated at 230° F. for 30 minutes after irradiation.

1 — unirradiated; 2 — 0.465 megarads; 3 — 0.93 megarads; 4 — 1.86 megarads; 5 — 3.72 megarads; 6 — 7.44 megarads; 7 — 14.88 megarads.

solutions (Table 2) and in the sugar-malic acid solution and an orange discoloration in the citric acid-sugar solutions (Table 5). Color intensity increased with increasing dosages, and was more pronounced in the unheated citric acid-sugar solutions than in the other unheated sugar-acid solutions. Interestingly enough, the more concentrated malic acid-sugar solutions (1:60; 30: 30) incurred less discoloration than the less concentrated ones (0.5:12; 6; 6) and the presence of 1.0% malic acid in the more concentrated sugar solutions did little to increase their darkening as compared to the unacidified sugar solutions. There was little difference in the behavior of the 3 sugars studied. Heating prior to irradiation caused only slightly greater darkening while heating after irradiation resulted in very appreciable darkening.

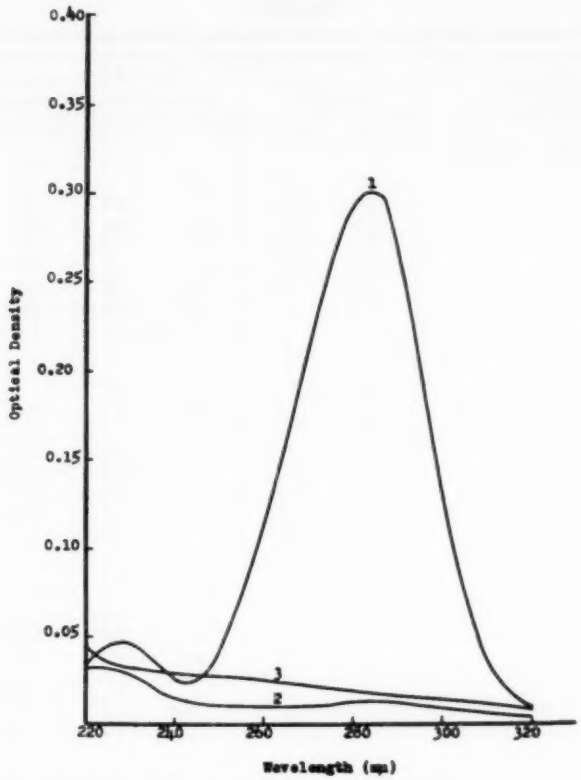

Figure 4. Effect of cathode ray irradiation on the ultraviolet absorption spectrum of aqueous 5-hydroxymethylfuraldehyde solutions.

1 — unirradiated; 2 — 0.93 megarads; 3 — 14.88 megarads.

This appreciable discoloration due post-irradiation heating indicates that colorless intermediates are formed in irradiation that are capable of producing brown-colored end products upon subsequent exposure to heat. Neither heat alone, nor irradiation alone produced the degree of discoloration that resulted from irradiation followed by heating.

Gamma ray irradiation generally had a somewhat more severe effect than did cathode rays in all systems studied. Whether this may have been due to errors in dosimetry, or to physical differences between the two types of irradiation is not known.

The pure sugar solutions underwent increases in free and total acidity as a result of irradiation (Table 3) the level of acidity increasing with increasing dosages. Irradiated glucose solutions yielded more acid than either of the other two sugars, resulting in approximately twice the titratable acid production at dosages of 0.93 to 14.88 megarads. Heating the solutions

TABLE 2

Effects of irradiation on the darkening of sugar solutions

Irradiation Dose (Megarads)	Optical Density at 450 mμ					
	Sucrose 60%		Fructose (30%)		Glucose (30%)	
	Cathode	Gamma	Cathode	Gamma	Cathode	Gamma
Unheated sugar solutions						
0	.007	.008	.005	0	0	0
0.465	.008	.012	.005	.004	.002	.002
0.93	.008	.012	.007	.008	.005	.005
1.86	.010	.003	.008	.010	.004	.005
3.72	.022	.025	.017	.021	.017	.022
7.44	.047	.028	.047	.026	.051	.062
14.88	.088	.107	.074	.088	.084	.100
Sugar solutions heated before irradiation						
0	.007	.003	.006	.003	0	0
0.465	.012	.017	.008	.005	.002	0
0.93	.013	.014	.010	.013	.002	.005
1.86	.027	.029	.014	.020	.008	.008
3.72	.032	.038	.022	.015	.014	.020
7.44	.059	.076	.062	.069	.031	.063
14.88	.148	.194	.106	.126	.099	.116
Sugar solutions heated after irradiation						
0	0	0	0	0	0	0
0.465	.027	.027	.005	.005	.055	.058
0.93	.058	.064	.040	.048	.065	.076
1.86	.100	.115	.069	.092	.108	.128
3.72	.173	.216	.142	.171	.168	.218
7.44	.382	.486	.323	.410	.367	.479
14.88	.815	1.05	.625	.733	.749	.997

prior to or after irradiation influenced the free acid production more than the total acidity.

Considerable increases in light absorption in the ultraviolet range occurred as a result of irradiation of the sugar solutions. It will be noted in Table 4, that, although initial maxima at 270 mμ were observed in the unheated samples, there occurred, as a result of irradiation, shifts to maxima at 265 mμ.

The sugar solutions heated prior to irradiation showed shifts from 270 or 275 mμ to 265, while heating after irradiation caused shifts in maxima from 265-270 mμ to 275 mμ. The unheated sugar-acid solutions and the solutions heated prior to irradiation which showed no initial absorption or maxima at 285 mμ though, in certain instances, also at 270 or 275, underwent shifts to maxima at 260-265 mμ. The sugar-acid solutions heated after irradiation had maxima, at the higher dosages at 270-280 mμ.

It would appear from the nature of these absorption changes that radiation-induced ultraviolet absorbing degradation products are quite different from heat-induced ultraviolet absorbing degradation products. Furthermore, heat is destructive to the radiation products, while irradiation destroys or alters the thermal degradation products. While these data do not as yet permit speculation on the reactants involved, it is clear that reactive com-

TABLE 3

Effects of gamma ray irradiation on the pH and titratable acidity of sugar solutions

Irradiation Dose (Megarads)	pH			Titratable Acidity [1]		
	Sucrose (60%)	Fructose (30%)	Glucose (30%)	Sucrose (60%)	Fructose (30%)	Glucose (30%)
Unheated sugar solution						
0	5.90	4.19	5.05	0.3	0.4	0.4
0.465	3.80	3.53	3.35	0.8	1.0	1.2
0.93	3.68	3.49	3.22	1.1	1.2	2.0
3.72	3.40	3.36	3.08	3.1	3.0	5.9
14.88	3.19	3.06	2.82	9.6	9.1	22.7
Sugar solution heated for 30 minutes before irradiation						
0	5.00	3.69	3.85	0.4	0.4	0.4
0.465	3.39	3.48	3.20	0.9	1.0	1.3
0.93	3.29	3.35	3.08	1.1	1.5	1.9
3.72	2.93	3.24	2.78	2.9	3.4	5.8
14.88	2.59	2.85	2.52	10.8	11.5	20.4
Sugar solutions heated for 30 minutes after irradiation						
0	5.35	3.95	3.90	0.3	0.3	0.3
0.465	3.30	3.60	3.20	0.8	1.1	1.4
0.93	3.18	3.27	2.95	1.2	1.6	1.8
3.72	2.88	3.00	2.65	3.1	3.7	5.9
14.88	2.49	2.65	2.42	11.0	11.8	20.5

[1] Ml. of 0.01234 N NaOH required to neutralize 5 ml. of sample.

pounds other than those associated with the heat "browning" of sugar systems (i.e. 5-hydroxymethylfurfural) must be produced from the sugars in irradiation and may be responsible for subsequent darkening.

An inflammable gas, presumed to be hydrogen, was produced by the various solutions upon irradiation. Analysis by gas chromatography, using a Model 154 B Vapor Fractometer,[k] confirmed the identity of the gas as hydrogen.

Effects of irradiation on pectin gels. Effects generally similar to those observed in sugar-acid solution were noted when pectin gel samples were irradiated (Table 7). No visible discoloration occurred in samples irradiated up to the 3.72 megarad level. At the 7.44 and 14.88 megarad levels, the samples developed a yellow color. Gels heated at 230° F. for 30 minutes prior to irradiation underwent a slight bleaching upon exposure to cathode rays at dosage of up to 3.72 megarads. Marked discoloration occurred in samples heated after irradiation. Absorption maxima in the ultraviolet range shifted from 285 to 265 with increasing dosages in "unheated" gels and gels heated for 30 minutes at 230° F. prior to irradiation (Figures 1 and 2). Heating the gels after irradiation resulted in a shift of the wavelength of maximum absorption to 280-285 mμ (Figure 3). Concurrently with these changes, there occurred also a marked reduction in gel strength with increasing dosages culminating in complete liquefaction at the higher dosages. An ignitable gas, i. e., hydrogen) was again detected. The gas appeared as small

[k] Manufactured by the Perkin-Elmer Corporation, Norwalk, Conn.

TABLE 4

Effects of irradiation on the wavelength of maximum ultraviolet absorption of sugar solutions

Irradiation Dose (Megarads)	λ Maximum (mμ)					
	Sucrose (60%)		Fructose (30%)		Glucose (30%)	
	Cathode	Gamma	Cathode	Gamma	Cathode	Gamma
Unheated sugar solutions						
0	None	None	270	275	None	None
0.465	270	265	265	265	None	None
0.93	265	260	265	265	265	260
1.86	265	265	265	265	265	260
14.88	265	265	265	265	265	265
Sugar solutions heated before irradiation						
0	275	None	270	270	None	None
0.465	265	260	265	270	None	None
0.93	265	265	265	265	None	None
1.86	265	265	265	265	265	265
14.88	265	265	265	265	265	265
Sugar solutions heated after irradiation						
0	None	None	None	None	None	None
0.465	None	None	None	None	None	265
0.93	270	270	275	270	270	265
1.86	270	275	270	275	275	275
14.88	275	270	275	270	275	270

bubbles entrapped in the gel at the dosages below which liquefaction took place (i.e., below 7.32 megarads), the occurrence of bubbles increasing with increasing dosages.

Effect of irradiation on apple sauce. An orange discoloration appeared in samples irradiated at all dosages, the intensity of discoloration increasing with increasing irradiation doses (Table 8). No appreciable changes in pH or titratable acidity due to irradiation took place in the unsweetened or sweetened apple sauces, or apple sauce filtrate, though the irradiation did markedly increase the acidity of apple sauce pomace, the extent of this effect appearing to be a function of the applied dose. Heating for 30 minutes at 212° F. after irradiation severely increased the orange discoloration. The viscosity decreased with increasing irradiation doses. This observation is in accordance with the findings of Kertesz and his associates *(3, 4, 6)* on the effects of irradiation on the viscosity of pectins and cellulose and on the viscosity of apple tissue.

It was noted the viscosity of the sweetened samples remained higher than that of the unsweetened apple sauce. Apple sauce pomace appeared to be more susceptible to discoloration than the apple sauce filtrate. Absorption maxima at 265 to 275 mμ were observed for irradiated samples other than those heated after irradiation in which maxima of 270 to 285 were observed. Gas was also produced in these samples.

Effect of irradiation on HMF. The shift in wavelength of maximum ultaviolet absorption from 285 mμ to 260-265 mμ which occurred in most

TABLE 5

Effects of gamma ray irradiation on the darkening of sugar-acid solutions

Irradiation Dose (Megarads)	Optical Density at 450 mμ								
	Sucrose			Fructose			Glucose		
	Unheated	Heated before irrad.	Heated after irrad.	Unheated	Heated before irrad.	Heated after irrad.	Unheated	Heated before irrad.	Heated after irrad.
Sugar-malic acid solutions (12; 6; 6:0.5)[1]									
0	.005	.006	.007	.002	.005	.007	.001	0	0
3.72	.087	.048	.324	.028	.051	.410	.068	.047	.398
7.44	.134	.130	.784	.119	.092	.620	.113	.114	.604
14.88	.228	.234	1.12	.221	.300	1.38	.198	.305	1.23
Sugar-malic acid solutions (60; 30; 30:1) [2]									
0	.013	.036	.024	0	.027	.020	.020	.027	.008
0.465	.012	.043	.122	.008	.025	.060	.002	.027	.052
1.86	.022	.083	.376	.022	.058	.284	.008	.046	.252
7.44	.071	.158	1.18	.058	.131	.98	.027	.063	.740
14.88	.140	.168	2.3	.097	.184	2.0	.071	.110	1.52
Sugar-citric acid solutions (60; 30; 30:1) [2]									
0	.018	.027	.026	.002	.018	.020	.008	.008	.012
0.465	.032	.058	.134	.029	.048	.094	.020	.021	.062
1.86	.087	.122	.388	.106	.086	.260	.059	.056	.260
7.44	.247	.238	1.23	.284	.195	.940	.164	.188	.92
14.88	.388	.276	2.2	.457	.293	1.63	.252	.248	1.52

[1] Solution containing 12% sucrose, 6% fructose or 6% glucose in 0.5% malic acid.
[2] Solutions containing 60% sucrose, 6% fructose, [illegible]

TABLE 6

Effects of gamma ray irradiation on the wavelength of maximum utraviolet absorption of sugar-acid solution

Irradiation Dose (Megarads)	Maximum (mμ)								
	Sucrose			Fructose			Glucose		
	Unheated	Heated before irrad.	Heated after irrad.	Unheated	Heated before irrad.	Heated after irrad.	Unheated	Heated before irrad.	Heated after irrad.
Sugar-malic acid solutions (12; 6; 6:0.5) [1]									
0	None	285	285	280	285	285	None	None	None
3.72	265	265	275	265	265	275	265	265	270
7.44	265	265	275	265	265	275	265	265	270
14.88	265	265	270	265	265	270	265	265	270
Sugar-malic acid solutions (60; 30; 30:1) [2]									
0	275	285	285	270	285	285	None	None	None
0.465	260	285	285	275	285	285	None	None	275
1.86	260	285	285	255	285	285	260	260	——
7.44	260	280	280	255	280	280	260	260	270
14.88	260	265	275	260	260	280	260	260	270
Sugar-citric acid solutions (60; 30; 30:1) [2]									
0	275	285	285	280	285	285	None	None	None
0.465	270	285	285	275	285	285	265	None	270
1.86	265	285	285	270	285	285	265	265	270
7.44	265	275	280	265	270	280	265	265	270
14.88	265	265	275	265	265	280	265	265	270

[1, 2] See footnotes Table 5.

TABLE 7

Effects of irradiation on the darkening and pH of pectin gels [1]

Irradiation Dose (Megarads)	Optical density at 450 mμ		pH	
	Cathode	Gamma	Cathode	Gamma
Unheated gels				
0	.031	.051	2.82	3.00
0.465	.027	.036	2.77	3.00
1.86	.022	.043	2.77	2.85
7.44	.060	.060	2.80	2.70
14.88	.063	.094	2.80	2.80
Gels heated before irradiation				
0	.061	.056	2.80	2.80
0.465	.034	.058	2.60	2.90
1.86	.046	.059	2.70	2.63
7.44	.058	.062	2.85	2.75
14.88	.056	.069	2.85	2.80

[1] Diluted to 30% solids prior to measurement.

products as a result of irradiation, appeared at first to suggest the destruction, upon irradiation of HMF whose characteristic maximum generally occurs near 285 mμ. To determine whether the substance(s) absorbing at 260-265 mμ originated from the HMF, pure solutions of the latter were irradiated (Figure 4). The disappearance of ultraviolet absorption, coupled with the fact that in many instances absorption at 260-265 mμ occurred upon irradiation even though no HMF (i.e. λ max. = 285 mμ) had been present initially, suggests that HMF is not the origin of the compound(s) absorbing at the lower wavelength. Nor does the HMF appear to be related to color development on irradiation, since pure solutions did not darken on exposure to cathode rays up to 3.72 megarads (Table 9). In this respect the irradiation induced discoloration of the systems studied differs from the thermal discoloration of these same systems which has been shown to involve HMF *(8, 9, 10, 16)*. Wertheim *et al. (17)* have recently attributed similar ultraviolet absorption at 265 mμ in irradiated milk to reductones. It would appear, from the present study, that the irradiation of HMF results in its breakdown to non-reactive acidic products. The identity of these products, as well as the identity of the reductones responsible for the absorption at 260-265 mμ are under further investigation.

SUMMARY AND CONCLUSIONS

The effects of gamma ray and cathode ray irradiation, at dosages ranging from 0.465–14.88 megarads, on sucrose, fructose and glucose solutions alone or in the presence of malic or citric acid, on pure sugar-acid-pectin gels, on apple sauce and on solutions of 5-hydroxymethylfurfuraldehyde (HMF) were studied.

Irradiation generally resulted in:

1. discoloration of a yellow, orange or brownish type, the severity of the discoloration being related to the magnitude of the dose.
2. increased acidity in the low acid products, i.e. sugar solutions, the tissue portion of the apple sauce (pomace), and HMF solutions.

TABLE 8

Effect of gamma irradiation on the viscosity, pH, acidity, darkening, and ultraviolet absorption of heated and unheated apple sauce [1]

Determination	Irradiation Dose (Megarads)	Sweetened Apple Sauce			Unsweetened Apple Sauce			Apple Sauce Pomace			Apple Sauce Filtrate		
		Unheated	Heated before irrad.	Heated after irrad.	Unheated	Heated before irrad.	Heated after irrad.	Unheated	Heated before irrad.	Heated after irrad.	Unheated	Heated before irrad.	Heated after irrad.
Viscosity (poise)	0	69.0	62.0	61.5	50.5	51.0	50.0	128.5	130.0	187.0	—	—	—
	1.86	26.0	30.0	23.5	25.0	25.0	23.0	116.0	114.0	100.0	—	—	—
	14.88	15.0	16.0	1.0	11.0	8.5	1.0	40.0	38.0	32.0	—	—	—
pH	0	3.25	3.20	3.27	3.20	3.15	3.18	4.00	3.70	3.50	3.35	3.29	3.31
	1.86	3.22	3.15	3.23	3.20	3.15	3.18	3.50	3.24	3.05	3.35	3.20	3.29
	14.88	3.20	3.12	3.15	3.20	3.16	3.18	2.95	2.78	2.69	3.35	3.20	3.28
Acidity [2]	0	2.1	2.0	2.0	2.0	2.0	2.0	0.8	0.9	0.9	1.9	2.0	2.0
	1.86	2.1	1.9	2.0	2.0	2.1	2.0	3.0	2.7	2.9	2.0	2.0	2.0
	14.88	2.2	2.2	2.1	2.0	2.2	2.1	13.9	13.1	13.5	2.2	2.2	2.1
Reflectance at 450 mμ (%)	0	11.2	11.8	10.9	14.5	15.5	10.9	15.0	14.0	14.0	15.5	14.5	14.0
	1.86	9.9	10.0	3.9	11.0	13.0	3.9	10.5	11.2	9.0	13.0	13.5	8.5
	14.88	8.2	8.5	1.6	6.1	7.5	1.6	7.5	7.0	2.9	9.2	9.8	2.0
λ max.[3] (mμ)	0	280	285	285	280	285	285	None	None	None	280	285	285
	1.86	275	275	285	275	275	280	275	265	270	275	270	275
	14.88	265	265	270	265	265	270	265	265	265	265	265	270

[1] Heated for 30 min. at 212° F.
[2] Ml. of 0.125 N NaOH required to neutralize 5 ml. filtrate of sample.
[3] Measured on filtrate of sample.

TABLE 9

Effect of cathode ray irradiation on the pH, titratable acidity, darkening and ultraviolet absorption of 0.1% aqueous 5-hydroxymethylfurfuraldehyde solutions

Irradiation dose (Megarads)	pH		Titratable Acidity [2]		Optical Density at 450 mμ		λ Max. (mμ)	
	Unheated	Heated [1]	Unheated	Heated	Unheated	Heated	Unheated	Heated
0	6.35	5.50	0.1	0.1	.018	.010	285	250
0.93	4.01	3.92	0.4	0.5	.002	.063	280	None
1.86	4.05	4.00	0.4	0.5	.002	.053	265	None
3.72	3.68	3.82	1.0	1.2	.018	.102	265	None
14.88	3.41	3.45	2.2	2.3	.034	.146	None	None

[1] Heated at 230° F. for 30 min. after irradiation.
[2] Ml. of 0.01234 N NaOH required to neutralize 5 ml. of solution.

3. a shift in the wavelength of maximum ultraviolet absorption toward 260-265 mμ, probably due to reductone formation. This shift is related to the disappearance of HMF when initially present but is not due to its breakdown products which are acid, non-colored and non-absorbing in the ultraviolet range.
4. loss of gelation in pectin gels and decreased viscosity of apple sauce.
5. formation of gas, (hydrogen).
6. marked browning and considerable ultraviolet absorption upon post-irradiation heating.

Irradiation-induced browning in the systems studied thus differs from heat-induced browning in that the former involves the formation of reductones, as intermediates, while the latter involves 5-hydroxymethylfurfural and other aldehydes, which appear to be destroyed by irradiation. The reductones, however, are capable of contributing materially to darkening when the systems are subjected to post-irradiation heating.

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TABLE 8

Effect of gamma irradiation on the viscosity, pH, acidity, darkening, and ultraviolet absorption of heated and unheated apple sauce [1]

Determination	Irradiation Dose (Megarads)	Sweetened Apple Sauce			Unsweetened Apple Sauce			Apple Sauce Pomace			Apple Sauce Filtrate		
		Unheated	Heated before irrad.	Heated after irrad.	Unheated	Heated before irrad.	Heated after irrad.	Unheated	Heated before irrad.	Heated after irrad.	Unheated	Heated before irrad.	Heated after irrad.
Viscosity (poise)	0	69.0	62.0	61.5	50.5	51.0	50.0	128.5	130.0	187.0	—	—	—
	1.86	26.0	30.0	23.5	25.0	25.0	23.0	116.0	114.0	100.0	—	—	—
	14.88	15.0	16.0	1.0	11.0	8.5	1.0	40.0	38.0	32.0	—	—	—
pH	0	3.25	3.20	3.27	3.20	3.15	3.18	4.00	3.70	3.50	3.35	3.29	3.31
	1.86	3.22	3.15	3.23	3.20	3.15	3.18	3.50	3.24	3.05	3.35	3.20	3.29
	14.88	3.20	3.12	3.15	3.20	3.16	3.18	2.95	2.78	2.69	3.35	3.20	3.28
Acidity [2]	0	2.1	2.0	2.0	2.0	2.0	2.0	0.8	0.9	0.9	1.9	2.0	2.0
	1.86	2.1	1.9	2.0	2.0	2.1	2.0	3.0	2.7	2.9	2.0	2.0	2.0
	14.88	2.2	2.2	2.1	2.0	2.2	2.1	13.9	13.1	13.5	2.2	2.2	2.1
Reflectance at 450 mμ (%)	0	11.2	11.8	10.9	14.5	15.5	10.9	15.0	14.0	14.0	15.5	14.5	14.0
	1.86	9.9	10.0	3.9	11.0	13.0	3.9	10.5	11.2	9.0	13.0	13.5	8.5
	14.88	8.2	8.5	1.6	6.1	7.5	1.6	7.5	7.0	2.9	9.2	9.8	2.0
λ max.[3] (mμ)	0	280	285	285	280	285	285	None	None	None	280	285	285
	1.86	275	275	285	275	275	280	275	265	270	275	270	275
	14.88	265	265	270	265	265	270	265	265	265	265	265	270

[1] Heated for 30 min. at 212° F.
[2] Ml. of 0.125 N NaOH required to neutralize 5 ml. filtrate of sample.
[3] Measured on filtrate of sample.

TABLE 9

Effect of cathode ray irradiation on the pH, titratable acidity, darkening and ultraviolet absorption of 0.1% aqueous 5-hydroxymethylfurfuraldehyde solutions

Irradiation dose (Megarads)	pH		Titratable Acidity [2]		Optical Density at 450 mμ		λ Max. (mμ)	
	Unheated	Heated [1]	Unheated	Heated	Unheated	Heated	Unheated	Heated
0	6.35	5.50	0.1	0.1	.018	.010	285	280
0.93	4.01	3.92	0.4	0.5	.002	.063	280	None
1.86	4.05	4.00	0.4	0.5	.002	.053	265	None
3.72	3.68	3.82	1.0	1.2	.018	.102	265	None
14.88	3.41	3.45	2.2	2.3	.034	.146	None	None

[1] Heated at 230° F. for 30 min. after irradiation.
[2] Ml. of 0.01234 N NaOH required to neutralize 5 ml. of solution.

3. a shift in the wavelength of maximum ultraviolet absorption toward 260-265 mμ, probably due to reductone formation. This shift is related to the disappearance of HMF when initially present but is not due to its breakdown products which are acid, non-colored and non-absorbing in the ultraviolet range.
4. loss of gelation in pectin gels and decreased viscosity of apple sauce.
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Irradiation-induced browning in the systems studied thus differs from heat-induced browning in that the former involves the formation of reductones, as intermediates, while the latter involves 5-hydroxymethylfurfural and other aldehydes, which appear to be destroyed by irradiation. The reductones, however, are capable of contributing materially to darkening when the systems are subjected to post-irradiation heating.

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THE INFLUENCE OF LEVEL OF IRRADIATION, TEMPERATURE AND LENGTH OF STORAGE UPON THE LEVEL OF CERTAIN CHEMICAL COMPONENTS AND PANEL SCORES FOR PRECOOKED BEEF, PORK AND VEAL [a, b, c]

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Irradiation of certain meat products produces characteristic objectionable flavors and odors. It has been assumed that the objectionable flavors are due to certain chemical changes, and a number of workers *(2, 3, 6, 8, 13)* have reported values for various chemical components both before and after irradiation. Recently it has been shown that the level of hydrogen sulfide, methyl mercaptan and carbonyls appear to be closely associated with the poor acceptability of irradiated beef, pork and veal *(10)*. However, data are not available in the literature showing the influence of temperature and length of storage upon either the chemical components or panel scores.

Consequently, this study was designed to ascertain the effects of length and temperature of storage upon panel scores, carbonyl compounds, hydrogen sulfide, methyl mercaptan, total glutathione, reduced glutathione and the pH of precooked irradiated meats.

EXPERIMENTAL PROCEDURE

Preparation and irradiation. The beef, pork and veal were prepared, cooked and irradiated at either 1.86×10^6 rads [d] or 2.79×10^6 rads as described in a previous publication *(10)*.

Storage. All samples irradiated at 1.86×10^6 rads were stored at 2° C. in order to prevent the growth of botulinum organisms. The samples irradiated at the higher level (2.79×10^6 rads) were stored at 2° C. and 24° C. for the beef and at 2° C., 24° C. and 38° C. for both pork and veal. Storage for both panel and chemical testing was for 0, 1, 3 and 6 months. At each of these storage periods, duplicate samples were removed and one was used for taste panel evaluation while the other was used for chemical determinations. Additional samples were available for panel ratings but not for chemical analysis at 12 months for beef and at 9 months for pork.

Panel ratings. Samples were prepared and presented to a consumer type panel of 18 judges, who rated the samples on the 9-point hedonic scale *(12)*. Although some judges

[a] Journal Article *2326* Michigan Agricultural Experiment Station, East Lansing.
[b] Journal Paper No. *171* American Meat Institute Foundation.
[c] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned number *961* in the series of papers approved for publication.
[d] A rad is 93/100 of a rep. It was recommended as the measure of radiation dosage by the International Congresses of Radiology in 1953 and 1956 and officially adopted by the QM Research and Engineering Command, July 1957.

participated in all panels, no effort was made to standardize panel members. Details of this phase of work have been previously reported *(10)*.

Chemical analysis. Samples were prepared and chemical analyses were made for hydrogen sulfide *(8)*, methyl mercaptan *(13)*, acid-salt soluble carbonyls *(3, 8)*, total glutathione *(4)*, reduced glutathione *(5)* and pH *(1)*.

Statistical treatment. The data were analyzed statistically by analysis of variance according to the procedure described by Snedecor *(14)* to determine the influence of level of irradiation, length and temperature of storage.

RESULTS AND DISCUSSION

Acceptability studies. Taste panel results are summarized in table 1. Examination of the results with beef at 0 months storage shows that the control roasts were more acceptable ($P = <.01$) than the irradiated. Although the panel rating was higher for the beef roast irradiated at 1.86 megarads than for the roast irradiated at 2.79 megarads, the difference was not statistically significant. During storage there was an initial improvement in acceptability scores at 1 month, but thereafter, a gradual decline occurred at each succeeding test period until 12 months, when all samples showed an unexplainable increase. Although the reason for the increase is not apparent, it has been observed in other studies *(11)*. Although some variation in score is evident at different storage periods, which is apparently associated with irradiation level and/or storage temperature, the overall statistical analysis showed panel scores for beef did not differ significantly between temperatures of storage or levels of irradiation.

Taste panel results with pork (table 1) show that unirradiated control chops were preferred over both the lower ($P = <.01$) and higher ($P = <.05$) levels of irradiation at 0 months storage. However, the difference between the two levels of irradiation was not significant except after 9 months storage, and it is possible that the effect noted then was due to the combined effect of level of irradiation, with storage time and temperature. Although significant differences in panel scores occurred between various temperatures at the different storage intervals, examination of the means indicates there was no major temperatue effect. However, at the end of 9 months storage the lower holding temperatures were preferred ($P = <.01$), probably due to the loss of consistency reported earlier at higher temperatures *(9)*.

Panel acceptability ratings for veal (Table 1) at 0 months did not differ significantly between the two levels of irradiation or between the unirradiated controls and the lower level of irradiation, but the unirradiated controls were preferred over the samples irradiated at the higher level. After 1 month storage, the sample irradiated at 1.86×10^6 rads was preferred ($P = <.01$) over the sample irradiated at 2.79×10^6 rads and stored at 2° C., but not over similar samples stored at either 24 or 38° C. The mean panel score for the samples stored 3 months was significantly higher than the mean at either 1 or 6 months storage.

Carbonyl compounds. A summary of the changes in acid-salt soluble carbonyl compounds is given in Table 2. Examination of the data for all three types of meat shows considerable variation, but certain general trends are evident. Although the level for frozen unirradiated beef is missing, data

TABLE 1

The effect of level of irradiation, temperature and length of storage upon panel scores of precooked beef, pork and veal [1]

Treatment	Beef						Pork						Veal				
	0 mo.	1 mo.	3 mos.	6 mos.	12 mos.	Means	0 mo.[7]	1 mo.	3 mos.[8]	6 mos.[9]	9 mos.[10]	Means	0 mo.	1 mo.	3 mos.	6 mos.	Means
Control-cooked unirradiated	6.87 [2]	—	—	—	—	—	6.61	—	—	—	—	—	6.35 [11]	—	—	—	—
1.86 × 10 [6] rads-storage @ 2° C.	4.22	5.72	4.89	4.61	6.56 [4]	5.20	5.17	5.89	5.89	6.61	6.89	6.36	5.17	5.28 [12]	5.17	5.17	5.19
2.79 × 10 [6] rads-storage @ 2° C.	3.57 *	6.22	4.33	5.11	5.50 [5]	4.95	5.76 *	6.67	6.22	6.78	6.28	6.44	4.48 *	3.94	5.89	4.44	4.50
2.79 × 10 [6] rads-storage @ 24° C.	3.57 *	5.78	4.67	4.28	4.44 [5]	4.79	5.76 *	5.94	5.44	6.95	4.78	5.78	4.48 *	5.00	5.44	4.94	5.13
2.79 × 10 [6] rads-storage @ 38° C.	—	—	—	—	—	—	5.76 *	6.61	6.28	6.11	3.89	5.72	4.48 *	4.78	5.50	4.67	4.98
Means	3.90	5.91 [3]	4.63	4.67	5.50 [6]		5.46	6.28	5.96	6.61	5.46		4.82	4.75	5.50 [13]	4.80	

[1] Panel scores are recorded on the hedonic scale which makes ratings in terms of like and dislike as follows: 1 — dislike extremely; 2 — dislike very much; 3 — dislike moderately; 4 — dislike slightly; 5 — neither like nor dislike; 6 — like slightly; 7 — like moderately; 8 — like very much; 9 — like extremely.

[2] With beef at 0 months, the control was preferred (P = .01) over both levels of irradiation.

[3] With beef, mean for samples stored 1 month preferred (P = .01) over means after storage at either 3 or 6 months.

[4] At 12 months storage with beef, the lower level of irradiation was preferred (P = .01) over both samples irradiated at the higher level.

[5] At 12 months, the beef sample irradiated at 2.79 megarads and stored at 2° C. was preferred (P = .01) over the sample stored at 24° C.

[6] Mean for 12 months storage for beef preferred (P = .05) over 3 and 6 months storage means.

[7] At 0 months for pork, the unirradiated sample was preferred over both the lower (P = .01) and higher (P = .05) levels of irradiation.

[8] At 3 months the pork sample at 24° C. was rated lower (P = .05) than those at either 2° C. or 38° C.

[9] At 6 months the pork sample at 24° C. was preferred (P = .05) over the sample at 38° C.

[10] At 9 months both samples of pork stored at 2° C. were preferred (P = .01) over the samples stored at 24° C. or 38° C.

[11] At 0 months the unirradiated control veal roast was preferred (P = .01) over all samples irradiated at 2.79 megarads.

[12] At 1 month storage, the veal sample irradiated at 1.86 megarads was preferred (P = .01) over the sample irradiated at 2.79 megarads and stored at 2° C.

[13] Mean of samples of veal stored for 3 months was preferred (P = .05) over the means for either 1 or 6 months storage.

* Score at 0 months for each storage temperature is duplication for use in following changes in acceptability.

TABLE 2

Effect of irradiation level, temperature and length of storage on the level of acid-salt soluble carbonyls in beef, pork and veal ($\times 10^{-5}$ M/g.)

Treatment	Beef					Pork					Veal				
	0 mo.	1 mo.	3 mos.	6 mos.	Means	0 mo.	1 mo.	3 mos.	6 mos.	Means	0 mo.	1 mo.	3 mos.	6 mos.	Means
Frozen-unirradiated	—	—	—	—	—	0.92	—	—	—	—	2.99	—	—	—	—
Control-cooked unirradiated	2.80	—	—	—	—	2.00	—	—	—	—	3.19	—	—	—	—
1.86 × 10^6 rads-storage @ 2° C.	7.02	5.75	9.82	5.52	6.45	7.42	7.59	7.25	6.21	7.12	7.13	6.21	8.05	5.51	6.72
2.79 × 10^6 rads-storage @ 2° C.	9.09 *	7.70	9.20	8.17	8.54	9.44 *	7.65	7.22	5.75	7.51	10.12 *	7.82	11.04	6.54	8.88
2.79 × 10^6 rads-storage @ 24° C.	8.63 *	4.80	8.46	3.91	7.03	9.49 *	4.57	3.43	3.86	5.34	9.66 *	4.97	10.35	4.72	7.42
2.79 × 10^6 rads-storage @ 38° C.	—	—	—	—	—	11.61 *	4.76	4.18	4.03	6.14	9.13 *	8.00	8.51	5.86	7.88
Means	8.25 [1]	6.08	9.16 [2]	5.87	—	9.49 [3]	6.14	5.52	4.96	—	9.01 [4]	6.75	9.49 [5]	5.66	—

[1] Mean for beef samples stored 0 months contained a greater amount ($P = <.05$) of carbonyls than mean for samples stored 6 months.
[2] Mean for sample of beef stored 3 months contained a significantly greater amount ($P = <.05$) of carbonyls than mean for samples stored 1 and 6 months.
[3] The greater amount of carbonyls at 0 months storage for pork was significantly higher ($P = <.05$) than for any storage period.
[4] Means for veal samples stored 0 months contained a greater amount of carbonyls than those at 1 ($P = <.05$) and 6 months ($P = <.01$).
[5] Veal samples stored 3 months contained a greater amount of carbonyls (1% level) than those stored for 1 and 6 months.

* Values at 0 month storage at different storage temperatures for samples irradiated at 2.79 megarads is due to chance allotment of sample to this series. Thus, some indication of the variability is evident.

for pork and veal indicates that on cooking alone there was an increase in the level of carbonyls. However, upon irradiation of the cooked meat, there was a marked increase in the amount of carbonyls. Furthermore, the level of carbonyls at 0 months storage was higher in every case for the meat irradiated at 2.79 megarads than for that irradiated at 1.86 megarads. Thus, it is evident that irradiation not only increased the amount of carbonyls, but that the increase was related to the level of treatment.

Examination of the means by storage temperatures reveals that samples stored at 2° C. after irradiation at 2.79 megarads had a consistently higher level of carbonyls than samples irradiated at the same level but stored at the higher temperatures of storage (24 or 38° C.). However, there was no consistent difference between samples stored at the two higher temperatures.

The effect of length of storage upon carbonyls is not so evident. Nevertheless, the level of carbonyls at 6 months storage for all three kinds of meat was significantly lower than at 0 months. There was a regular decrease in carbonyls for each succeeding storage period with pork. With beef and veal there was a regular decline in the level of carbonyls, except at 3 months with both types of meat an unexplainable increase in carbonyls occurred.

Methyl mercaptan. The data on methyl mercaptan are summarized in Table 3. Although cooking did not appear to influence the amount of methyl mercaptan, there was a marked increase upon irradiation. However, there was no significant difference between the two levels of irradiation, between storage periods or between temperatures of storage. Interestingly enough, the level of methyl mercaptan in veal was nearly twice as high as in either pork or beef.

Hydrogen Sulfide. A summary of the data on hydrogen sulfide is presented in Table 4. Cooking of the unirradiated meat resulted in a marked increase in hydrogen sulfide, and a further increase occurred upon irradiation. However, the differences in hydrogen sulfide content were not statistically significant between levels of irradiation, between storage periods or between temperatures of storage, with the exception of the fact that the mean for pork irradiated at 2.79 megarads and stored at 24° C. had a greater amount of hydrogen sulfide than the samples irradiated at either level and stored at 2° C.

Total glutathione. Results on total glutathione are summarized in Table 5. Since the beef was not analyzed for this component, values are given for only pork and veal. Examination of the data reveals that a marked decrease occurred upon cooking the unirradiated meat, but there did not appear to be any further change upon irradiation. The means for storage temperatures indicate that there was some further breakdown of total glutathione at storage temperatures of 24 and 38° C., but at 2° C. the level remained relatively constant. With pork, there was a gradual decline in total glutathione as storage progressed, and although this was true with veal for 0, 1 and 3 months, at 6 months the highest mean value for any storage period was obtained. The reason for the high value at 6 months storage is not apparent.

Reduced glutathione. Values for reduced glutathione are presented in Table 6. As with total glutathione there was a decrease in reduced glutathione upon cooking unirradiated pork and veal, while the frozen unirradiated beef

TABLE 3

The effect of level of irradiation, temperature and length of storage upon the level of methyl mercaptan in beef, pork and veal (gamma/g.)

Treatment	Beef					Pork					Veal				
	0 mo.	1 mo.	3 mos.	6 mos.	Means	0 mo.	1 mo.	3 mos.	6 mos.	Means	0 mo.	1 mo.	3 mos.	6 mos.	Means
Frozen-unirradiated	—	—	—	—	—	0.13	—	—	—	—	0.40	—	—	—	—
Control-cooked unirradiated	0.21	—	—	—	—	0.15	—	—	—	—	0.41	—	—	—	—
1.86×10^6 rads-storage @ 2° C.	0.45	0.44	0.23	0.30	0.36	0.30	0.25	0.47	0.35	0.34	0.53	0.95	0.55	0.75	0.70
2.79×10^6 rads-storage @ 2° C.	0.48 [1]	0.40	0.37	0.23	0.37	0.30 [1]	0.45	.0.34	0.20	0.32	0.66 [1]	0.67	1.16	0.65	0.78
2.79×10^6 rads-storage @ 24° C.	0.56 [1]	0.46	0.44	0.47	0.48	0.57 [1]	0.56	0.47	0.25	0.46	1.30 [1]	0.60	1.05	0.95	0.98
2.79×10^6 rads-storage @ 38° C.	—	—	—	—	—	0.47 [1]	0.45	0.50	0.45	0.47	0.80 [1]	0.08	0.73	1.05	0.66
Means	0.50	0.43	0.35	0.33	—	0.41	0.43	0.44	0.31	—	0.82	0.58	0.87	0.85	—

[1] Values at 0 month storage for samples irradiated at 2.79 megarads is due to analysis by allotment of several samples. Results are not due to temperature effects but provide information on variability.

TABLE 4

The effect of level of irradiation, temperature and length of storage upon the level of Hydrogen Sulfide in beef, pork and veal (gamma/g.)

Treatment	Beef					Pork					Veal				
	0 mo.	1 mo.	3 mos.	6 mos.	Means	0 mo.	1 mo.	3 mos.	6 mos.	Means	0 mo.	1 mo.	3 mos.	6 mos.	Means
Frozen-unirradiated	—	—	—	—	—	0.00	—	—	—	—	0.10	—	—	—	—
Control-cooked unirradiated	0.66	—	—	—	—	1.17	—	—	—	—	0.85	—	—	—	—
1.86×10^6 rads-storage @ 2° C.	1.04	0.52	0.70	0.98	0.81	2.04	1.71	1.50	1.74	1.75	1.95	2.42	0.78	1.38	1.63
2.79×10^6 rads-storage @ 2° C.	1.20 [2]	0.003	0.78	1.40	0.84	1.19 [2]	2.54	0.60	1.86	1.55	2.38 [2]	2.58	1.25	1.75	1.99
2.79×10^6 rads-storage @ 24° C.	1.18 [2]	1.16	1.58	3.24	1.79	2.86 [2]	3.13	2.70	2.50	2.80 [1]	2.46 [2]	1.24	0.88	1.79	1.59
2.79×10^6 rads-storage @ 38° C.	—	—	—	—	—	2.78 [2]	2.26	0.92	2.90	2.22	2.27 [2]	0.28	2.37	1.42	1.58
Means	1.14	0.56	1.02	1.87	—	2.22	2.41	1.43	2.25	—	2.26	1.63	1.32	1.58	—

[1] Mean of pork samples irradiated at 2.79×10^6 rads and stored at 24° C. contained a greater amount ($P = <.05$) of hydrogen sulfide than those at either level of irradiation and stored at 2° C.

[2] See footnote [1] table 3.

TABLE 5

The effect of level of irradiation, temperature and length of storage upon the level of total glutathione in pork and veal (mgs/100 gs.)

Treatment	Pork					Veal				
	0 mo.	1 mo.	3 mos.	6 mos.	Means	0 mo.	1 mo.	3 mos.	6 mos.	Means
Frozen-unirradiated	28.25	—	—	—	—	17.50	—	—	—	—
Control-cooked unirradiated	16.19	—	—	—	—	10.69	—	—	—	—
1.86×10^6 rads-storage @ 2° C.	13.75	10.38	13.25	11.50	12.22	16.25	12.25	4.75	14.87	12.03 [2]
2.79×10^6 rads-storage @ 2° C.	12.50 [5]	14.00	12.00	9.50	12.00	10.75 [5]	12.37	8.00	14.37	11.37 [2]
2.79×10^6 rads-storage @ 24° C.	14.36 [5]	10.00	7.37	5.25	9.24	8.00 [5]	7.50	4.25	11.37	7.78
2.79×10^6 rads-storage @ 38° C.	12.62 [5]	9.25	7.87	5.00	8.68	9.25 [5]	7.50	4.50	6.13	6.84
Means	13.16 [1]	11.08	9.08	6.58	—	11.06 [4]	9.90 [3]	5.38	11.68 [4]	—

[1] The mean for pork samples stored 0 months contained a greater amount ($P = <.05$) of total glutathione than samples stored 3 and 6 months.

[2] Means of the two levels of irradiation for veal stored at 2° C. contained a greater amount ($P = <.05$) of total glutathione than means of samples at higher level of irradiation and stored at 24° and 38° C.

[3] Mean of veal samples stored 1 month contained a greater amount ($P = <.05$) of total glutathione than mean of samples stored 3 months.

[4] Mean of veal samples stored 0 and 6 months contained a greater amount ($P = <.01$) than mean of samples stored 3 months.

[5] See footnote [1] Table 3.

TABLE 6

The effect of level of irradiation, temperature and length of storage upon the level of reduced glutathione in beef, pork and veal (mgs./100 gms.)

Treatment	Beef					Pork					Veal				
	0 mo.	1 mo.	3 mos.	6 mos.	Means	0 mo.	1 mo.	3 mos.	6 mos.	Means	0 mo.	1 mo.	3 mos.	6 mos.	Means
Frozen-unirradiated	—	—	—	—	—	8.00	—	—	—	—	4.25	—	—	—	—
Control-cooked unirradiated	14.28	—	—	—	—	5.57	—	—	—	—	2.06	—	—	—	—
1.86 × 10^6 rads-storage @ 2° C.	13.13	14.75	9.50	11.50	12.22[2]	6.13	6.25	5.50	2.50	5.10[3]	3.50	5.50	0.00	6.37	3.84
2.79 × 10^6 rads-storage @ 2° C.	8.50[7]	10.75	4.87	6.75	7.72[2]	4.13[7]	7.50	4.00	1.25	4.22	2.25[7]	6.37	0.00	7.25	3.97
2.79 × 10^6 rads-storage @ 24° C.	8.50[7]	5.38	2.00	1.50	4.34	5.38[7]	4.50	3.13	0.75	3.44	1.50[7]	3.37	0.00	0.00	1.22
2.79 × 10^6 rads-storage @ 38° C.	—	—	—	—	—	4.00[7]	4.25	3.50	0.75	3.12	3.25[7]	1.75	0.00	1.50	1.63
Means	10.04[1]	10.29[1]	5.46	6.58	—	4.91	5.62	4.03[4]	1.31[5]	—	2.62	4.25	0.00[6]	3.78	—

[1] Mean of beef samples stored 0 and 1 months contained a greater amount ($P = <.05$) of reduced glutathione than those stored 3 and 6 months.

[2] Mean of beef samples at lower level of irradiation contained a greater amount ($P = <.01$) of reduced glutathione than samples with the higher level of irradiation stored at 2° and 24° C. Mean of samples of beef irradiated at 2.79 × 10^6 rads and stored at 2° C. contained a greater amount ($P = <.05$) of reduced glutathione than samples stored at 24° C.

[3] The mean of pork samples at the lower level of irradiation stored at 2° C. contained a significantly greater amount ($P = <.05$) of reduced glutathione than the samples with higher level of irradiation and stored at 24° and 38° C.

[4] Mean of pork samples stored 3 months contained significantly less ($P = <.05$) reduced glutathione than the mean of samples stored 1 month.

[5] Mean of pork samples stored 6 months contained significantly less ($P = <.01$) reduced glutathione than those at any other storage period.

[6] Mean of veal samples stored 3 months had significantly less ($P = <.05$) reduced glutathione than samples stored 1 and 6 months.

[7] See footnote [1] table 5.

sample was not analyzed. Although irradiation of the cooked meat at 1.86 megarads did not greatly alter the level of reduced glutathione, the level was consistently lower after irradiation at 2.79 megarads. Examination of the means by storage periods reveals a significant decrease in reduced glutathione for both beef and pork, but there was no clear-cut trend for veal. With veal at 3 month storage, no reduced glutathione could be detected chemically in any of the samples. The reason for the low levels are not apparent. Means by storage temperatures were higher for the samples stored at 2° C. over those stored at the higher temperatures. Thus, results indicate that reduced glutathione is more stable during storage at 2° C. than at higher temperatures.

pH. A summary of pH values is given in Table 7. It is evident that cooking the unirradiated meat resulted in a marked increase in pH values. Although considerable variation occurred between pH values for individual samples during storage, length of storage did not appear to be a factor. Even though level of irradiation *per se* did not appear to influence pH values, the means for the samples irradiated at 1.86 megarads and stored at 2° C. were slightly but consistently lower than those irradiated at 2.79 megarads and stored at the same temperature. With beef, the lower level of irradiation resulted in a significantly ($P = <.01$) lower pH than the higher level, but the difference was not significant for pork and veal. However, the mean pH values for pork and veal were lower when the samples were irradiated at the lower level (1.86 megarads).

SUMMARY

A study was conducted to ascertain the influence of level of irradiation, length and temperature of post-irradiation storage upon panel scores and the level of certain chemical components in precooked beef, pork and veal. Taste panel ratings showed the unirradiated samples were preferred over the irradiated. Although there was a general improvement in panel score during the shorter storage periods, differences between the two levels of irradiation were not significant. Furthermore, there was no measurable effect of temperature or length of storage upon panel score.

Although the methyl mercaptan content of meat was not greatly altered by cooking there was approximately a two-fold increase upon irradiation. However, level of irradiation, temperature and length of storage did not appear to alter the level. With hydrogen sulfide, there was a considerable increase from the raw to the cooked state and another marked increase upon irradiation. On the other hand, level of irradiation, length and temperature storage were without effect upon the hydrogen sulfide content. Cooking alone resulted in an increase in carbonyl compounds, and a two to four-fold increase occurred upon irradiation with the raise being correlated with the level of irradiation. Furthermore, samples stored at 2° C. after irradiation at 2.79 megarads contained consistently higher levels of carbonyl compounds than samples irradiated at the same level but stored at higher temperatures.

The level of total glutathione was markedly lowered by cooking, but no change occurred upon irradiation. When stored at 2° C. there was no apparent change, but storage at both 24 and 38° C. resulted in some breakdown of total glutathione. Similar to total glutathione, the content of reduced glutathione was lowered upon cooking but remained essentially unchanged

TABLE 7

The effect of level of irradiation, temperature and length of storage upon pH in beef, pork and veal.

Treatment	Beef					Pork					Veal			
	0 mo.	1 mo.	3 mos.	6 mos.	Means	0 mo.	1 mo.	3 mos.	6 mos.	Means	0 mo.	1 mos.	3 mos.	Means
Frozen-unirradiated	—	—	—	—	—	5.90	—	—	—	—	5.55	—	—	—
Control-cooked unirradiated	6.30	—	—	—	—	6.33	—	—	—	—	6.05	—	—	—
1.86 × 10 [6] rads-storage @ 2° C.	5.80	5.80	5.75	6.00	5.84 [2]	6.20	5.95	5.90	6.15	6.05	6.30	6.00	6.30	6.20
2.79 × 10 [6] rads-storage @ 2° C.	6.28 [6]	6.15	6.10	6.20	6.18 [1]	6.20 [6]	6.10	6.00	6.30	6.15 [3]	6.30 [6]	6.30	6.20	6.27
2.79 × 10 [6] rads-storage @ 24° C.	6.25 [6]	6.40	6.30	6.40	6.34	6.20 [6]	5.95	5.90	6.20	6.06	6.38 [6]	6.20	6.15	6.22
2.79 × 10 [6] rads-storage @ 38° C.	—	—	—	—	—	6.20 [6]	5.95	5.90	6.30	6.09	6.32 [6]	6.10	6.15	6.21
Means	6.11	6.12	6.05	6.20	—	6.20 [5]	5.99 [4]	5.92	6.24 [5]	—	6.32	6.15	6.20	—

[1] Mean of beef samples irradiated at 2.79 megarads and stored at 2° C. significantly more acid ($P = <.05$) than mean of samples irradiated at 2.79 megarads and stored at 24° C.

[2] Mean pH of beef samples irradiated at 1.86 megarads significantly lower ($P = <.01$) than the means of samples irradiated at 2.79 megarads and stored at 2° and 24° C.

[3] Mean of pork samples irradiated at 2.79 megarads and stored at 2° C. significantly more neutral ($P = <.05$) than the mean of samples irradiated at 1.86 megarads and mean of samples irradiated at 2.79 megarads and stored at 24° C.

[4] Mean of pork samples stored 1 month significantly more neutral ($P = <.05$) than the mean of samples stored 3 months.

[5] Mean of pork samples stored 0 and 6 months significantly more neutral ($P = <.01$) than mean of samples stored 1 and 3 months.

[6] See footnote [1] Table 3.

when irradiated. However, the samples stored at 2° C. had less destruction of reduced glutathione than those stored at higher temperatures.

Cooking resulted in an increase in pH. Although level of irradiation did not appear to influence pH values, after storage the mean for the samples irradiated at the lower level and stored at 2° C. were more acidic than similarly treated samples irradiated at the higher level.

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EFFECT OF IRRADIATION ON QUALITY OF GROUND PORK AND OF COOKING CONVENTIONALLY AND ELECTRONICALLY

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The purpose of this study was to determine the effect of beta irradiation with 3×10^6 and 6×10^6 rep, and with 6×10^6 rep plus monosodium glutamate on the palatability, and objective color, fat, peroxide, moisture, pH, and thiamine content of raw and cooked ground pork. Results of 3 methods of cooking were compared: electric test oven, electric skillet, and electronic oven. Retention of thiamine in electronic cooking was determined.

Thiamine is known to be one of the B-vitamins relatively susceptible to ionizing radiations *(1, 6, 12)*. The mechanism of thiamine destruction in irradiated food is not known. Groninger *et al. (7)* postulated that oxygen is probably not required for destruction.

Pork was chosen for study because it has the highest thiamine content of any of the muscle meats and also, according to Huber *et al. (8)* because it develops less objectionable off-odors and flavors during radiation than do beef, lamb or veal.

Huber *et al. (8)*, Morgan *(13)*, Goldblith and Proctor *(6)* and Proctor *et al. (18)* reported that radiation of food in the frozen state lessened effects of radiation. On the other hand, Schultz *et al. (19)* found no significant difference in intensity of radiation flavor between meats irradiated while unfrozen and frozen. Huber *et al. (8)* found that radiation of meats in the frozen state did not interfere with degree of sterilization and enzyme inhibition.

EXPERIMENTAL

History of meat. Pork Boston butts, purchased from the open market, were ground twice in a commercial electric meat grinder at the market. The pork was shaped, wrapped, and sealed in moisture vapor-resistant cellophane by a commercial machine into patties approx. ½ × 3¾ × 4¾ inches, weighing approx. ⅓ lb. each. The ½ in. thickness was necessary because of limited penetration of beta rays.

Packages were divided into four 40-lb. lots: (1) control (2) those assigned to treatment with (a) 3×10^6 rep, (b) 6×10^6 rep and (c) 6×10^6 rep after addition of monosodium glutamate. MSG, 0.66%, was incorporated by mixing the 40-lb. lot in an institutional electric mixer for 6 minutes. This procedure, which is common practice in preparing meat loaves in institution kitchens, incorporates air and breaks up the meat somewhat.

The control lot was frozen and stored in a home-freezer (− 20° F.). The rest of the packages were frozen, packed in dry ice, and shipped by express to Cambridge, Mass., where they were beta irradiated in a Van de Graaf accelerator.

Upon receipt of the radiated meat it was reshaped and repackaged in order to have the patties thick enough to obtain internal temperatures during cooking, and also to have them representative in shape and size of those commonly used in homes. This meant, however, exposure to oxygen of the air. Each lot was partially thawed to allow for mixing and shaping. Mixing was done to insure a better distribution of fat. Patties weighing 100 g. each were shaped in metal rings to 3 in. dia. and ⅞ in. thickness and placed directly on a metal cooky sheet and frozen. Each frozen patty was then rewrapped in Dupont

MSAT-87 cellophane, sealed, labeled, and returned to the freezer (− 20° F.). All patties were tested within 8 weeks.

Methods. *Sampling.* For sampling raw meat, 2 patties from each of the 4 lots were partially thawed in the sealed cellophane in the refrigerator. One patty was cut in half horizontally and one half was used for objective color determination. The 1½ remaining patties were ground twice, mixed well after each grinding and weighed for chemical analysis.

Cooked meat was sampled in a similar manner except the two grindings and mixings were omitted since they were unnecessary for complete extraction in chemical analysis.

Patties from the control lot and from each of the three irradiated lots were cooked on each testing day. Only one cooking method was used on any one day.

Cooking. All patties were cooked from the frozen state to a predetermined temperature in oven-glass pans. Special thermometers [a] made for use in electronic ovens were used since they respond more slowly to temperature changes than does the standard mercury thermometer. It is possible, therefore, that the meat may have been cooked to temperatures higher than those indicated.

Electric test oven. Patties from each of the 4 lots were placed in separate shallow pans, 2 in. deep and 8¼ in. diameter, and were placed on racks on the hearth of a well-insulated electric test oven [b] which had been preheated to 176.6° C. (350° F.). The 4 lots were cooked from the frozen state simultaneously to an internal temperature of 87.8° C. (190° F.).

Electric skillet. Patties from each lot were placed in separate electric skillets which had been adjusted to a temperature of 149° C. (300° F.). Temperature of the skillet was checked with a griddle thermometer. The frozen patties which had been placed in a cold skillet were cooked 10 min. on one side, then turned and cooked 10 min. on the other side, after which they were turned again. The patties were cooked to an internal temperature of 76.6° C. (170° F.). In preliminary studies in which they were cooked to 87.8° C. (190° F.) even at the low temperature of 149° C. (300° F.) the surface was too brown. At an internal temperature of 76.6° C. (170° F.) the centers of the patties looked and tasted as if they were done. Internal temperatures alone do not necessarily determine degree of doneness. Jacobson and Fenton *(9)* found that the color of beef muscles (somewhat cylindrically-shaped) cooked to the same internal temperature ranged from tan to a reddish pink depending upon the size of the muscle and therefore the cooking time.

Order of cooking the 4 lots each day was according to a statistical design.

Electronic oven. Five patties from each lot were placed in a circle in an oven-glass pan, 10¼ in. dia. and 1¼ in. deep. For more uniform cooking, no patty was placed in the center. To insert the thermometer in the meat easily, the hard-frozen patties were exposed in the uncovered pan to the "low" heat (input approx. 450 watts) of the institution electronic oven [c] for 1 min. after which each patty was rotated so that the inside half was turned to the outside of the pan, — the side of the patty next to the outer rim of the pan absorbed more of the waves and softened before the inner side. An oven-glass plate was inverted over the pan to prevent spattering. Patties were cooked to an internal temperature of 87.8° C. (190° F.). In preliminary studies, patties cooked to 85.0° C. (185° F.) had pinkish raw-looking areas in them. One lot was cooked at a time; order of cooking was according to the statistical design used for skillet cooking.

Palatability testing. After cooking, 0.7 g. NaCl was sprinkled on each of the 2 broad sides of each patty to be tested. Scoring was by 9 judges from the faculties of Animal Husbandry, Hotel School, School of Nutrition, Food and Nutrition, and Institution Management. Patties from the control lot and each of the 3 irradiated lots cooked by one of the 3 methods were sampled each testing day. Each judge was given a radial one-fourth section of one patty from each lot according to a randomized order.

Patties were scored on desirability for aroma with sniffing, color, flavor and juiciness on a scale from 0 to 10. A score of 5 was average. In preliminary testing it was observed that, although each lot had received the same grinding treatment, the radiated lots seemed to have a finer texture than the control. Therefore, judges were asked to score fineness of grind. Order of preference was also given.

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[a] Taylor Instrument Co.

[b] Despatch oven.

[c] RADARANGE, Raytheon Manufacturing Company, Waltham, Massachusetts.

Objective color determination. The "Rd" (intensity), "a" (redness) and "b" (yellowness) values of the inside cross section of the cooked patties (see sampling) were measured with the Hunter Color and Color Difference Meter. Color was reported as a/b ratio (hue).

Chemical analysis. Fat was determined by the Stansby method *(23)* with slight modification. Perioxide values were determined by the method of Stansby *(23)*, modified by Watts *et al. (27)* and Naumann *et al. (15)*.

Moisture: Approx. 5 g. of meat were dried at 70° F. under a vacuum of 27 in. for 24 hours.

Thiamine: Thiamine was extracted by the method of Watts *et al. (28)* with slight modification and was measured by the thiochrome method of Conner and Straub *(4)*. Results were reported on the dry, fat-free basis as well as on the moist, fat basis. Statistical analysis was made of the content on the dry, fat-free basis only.

Statistical analysis. Analysis of variance according to Snedecor *(22)* was used in the treatment of the data. Each study was repeated 4 times.

RESULTS AND DISCUSSIOSN

Subjective observations

Raw Meat. During mixing to better distribute the fat prior to reshaping the patties, several observations were made. Radiated meat felt softer and more moist to the hands than did the control. Water-holding capacity may have been decreased. Schweigert *et al. (20)* postulated that during radiation bound water undergoes some change which might account for the difference noted. Morgan *(14)* stated that liquefaction may be attributed to protein degradation due to radiation.

Melting point of the fat might also have been lowered. Scribney *et al. (21)* reported that results with gamma-irradiated fats suggested that with high radiation dosages some cleavage of fatty acid peroxides occurred with resultant formation of fatty acid split products. Cell structure of the meat might also have been broken during radiation allowing the fat to coalesce.

The radiated meat felt looser in texture and as if it had received a finer grind. Fat felt waxy and tended to cling to the hands. Some liquid fat remained in the bottom of the mixing bowl after the last portion of radiated meat was removed. None of these observations applied to control meat. Evidently some change had occurred in fat during radiation, possibly fatty acids had been formed. Waxy feeling might indicate formation of polymers of fat. Again breaking of cell structure might have been responsible.

The 6×10^6 rep lot to which MSG had been added prior to radiation appeared softest and moistest and also seemed somewhat porous. Air was incorporated during blending in the electric mixer so that there were 2 variables in this part of the study: addition of MSG and air. Also the meat was broken up somewhat in mixing.

Aroma of raw radiated meat was similar to that of lard cracklings.

Cooked Meat. Radiated patties flattened out somewhat during cooking — more so with increased radiation and with addition of MSG with accompanying incorporation of air and breaking up of the ground meat. The control, on the other hand, decreased in diameter and increased in height.

The cooked control had a relatively coarse, firm, solid texture; radiated meat had a fine, loose almost spongy texture. This difference was noticeable in cutting patties for palatability testing, and in crumbling and mixing for sampling.

Cooked radiated meat, like raw meat, looked and felt more moist than the cooked control lot.

Palatability. Radiation resulted in significantly lower scores for aroma, color of cut surface, flavor and juiciness of cooked patties (Tables 1, 2).

TABLE 1

Mean values for palatability scores of irradiated ground pork [1]

Quality factor	Pretreatment	Cooking Method			Pretreatment means
		Electric oven	Skillet	Electronic oven	
Aroma	Control	7.1	7.6	7.6	7.4
	3×10^6 rep	4.4	3.9	2.8	3.7
	6×10^6 rep	3.6	3.0	2.9	3.2
	6×10^6 rep with MSG	3.7	3.1	2.5	3.1
	Means	4.7	4.4	3.9	
Color	Control	6.5	7.0	7.6	7.0
	3×10^6 rep	6.8	6.4	5.3	6.2
	6×10^6 rep	6.3	6.4	5.0	5.9
	6×10^6 rep with MSG	6.6	6.1	5.5	6.1
	Means	6.5	6.5	5.8	
Flavor	Control	7.5	7.8	7.8	7.7
	3×10^6 rep	3.9	4.0	3.1	3.7
	6×10^6 rep	3.6	2.9	2.2	2.9
	6×10^6 rep with MSG	3.2	2.6	2.0	2.6
	Means	4.5	4.3	3.8	
Juiciness	Control	6.8	7.0	7.3	7.0
	3×10^6 rep	5.9	6.3	6.5	6.2
	6×10^6 rep	6.0	5.9	5.9	5.9
	6×10^6 rep with MSG	5.8	5.9	5.8	5.8
	Means	6.1	6.3	6.4	

[1] Patties weighing approx. 100 g., 3 in. dia. and ⅞ in. thickness. In the 2 ovens they were cooked to an internal temperature of 190° F. and in the skillet to 170° F. Patties from each of the 4 lots were cooked by one method on each of the testing days. Possible maximum score was 10.

It increased the apparent fineness of grind. Differences increased with increased radiation.

"Irradiation odor", which was strongest during cooking and when the meat was hot, diminished upon standing but could be detected when the meat reached room temperature.

"Off-odor" of radiated meats has been attributed to sulfur-containing compounds. Doty *(5)* found that less objectionable odor was developed in meat containing large amounts of fat than in meats containing less fat. Batzer *et al. (2)* stated that although products from fat in radiated meat do not contribute directly to "off-odors", meats with a high fat content do not develop "off-odors" to the same extent as do leaner meats. Carbonyl compounds formed by radiation of meat and meat fats may possibly have a role in decreasing apparent "off-odors" by reacting with compounds that do contribute, i.e., with sulfhydral compounds and amines *(2)*. It is well known that products first formed by radiation may not only break down further but also react with each other.

TABLE 2

Mean squares and significance of palatability of irradiated ground pork

Source of variance	d. f.	Palatability factors			
		Aroma	Color	Flavor	Juiciness
Pretreatments	3	52.64**	3.00**	65.68**	3.56**
3×10^6 vs. 6×10^6	1	1.82*	0.43	3.53**	0.54*
$3 \times 10^6 + 6 \times 10^6$ vs. 6×10^6 + MSG	1	0.89	0.02	3.29**	0.57*
Control vs. all irradiated	1	155.21**	8.56**	190.21**	9.56**
Cooking Methods	2	2.44**	2.34*	2.57**	0.24
Test oven vs. Electronic oven	1	4.80**	3.85*	4.81**	0.48*
Skillet vs. Electronic oven and Test oven	1	0.08	0.83	0.33	0.01
Cooking Method × Pretreatment	6	0.97*	1.75*	0.61	0.17
Test oven vs. Electronic oven × 3×10^6 vs. 6×10^6	1	0.81	0.05	0.20	0.56*
Test oven vs. Electronic oven × $3 \times 10^6 + 6 \times 10^6$ vs. 6×10^6 + MSG	1	0.00	0.20	0.01	0.12
Test oven vs. Electronic oven × Control vs. all irradiated	1	4.51**	9.07**	3.08**	0.21
Skillet vs. Ovens × 3×10^6 vs. 6×10^6	1	0.37	0.35	0.33	0.03
Skillet vs. Ovens × $3 \times 10^6 + 6 \times 10^6$ vs. 6×10^6 + MSG	1	0.02	0.46	0.05	0.03
Skillet vs. Ovens × Control vs. all irradiated	1	0.11	0.38	0.00	0.04

* Significant, at 5% level.
** Highly significant, at 1% level.

Radiation resulted in a paler color.

MSG did not improve the flavor of the 6×10^6 rep lot. How much effect the incorporation of air with MSG had, is not known. Proctor *et al. (17)* were unsuccessful in minimizing off-flavor in radiated beef slices with MSG even with little incorporation of air. Working with unirradiated ground pork, Noton *et al. (16)* and Kemp *(10)*, however, found that 0.1% MSG significantly increased the desirability of flavor of ground pork after freezer storage. The radiated meat in this study was not only not irradiated and stored in the absence of oxygen, but was exposed to the air during mixing and reshaping after radiation and during freezing.

No doubt higher aroma and flavor scores would have been obtained if the meat had been irradiated and stored in the absence of oxygen. Several laboratories *(5, 7, 8, 18)* have reported that organoleptic changes in meat were reduced when it was radiated in the absence of oxygen.

Work done at the Quartermaster Food and Container Institute for the Armed Forces *(25)* indicated that the radiated meat in the above study might have received higher aroma and flavor scores if it had been thawed and kept at room temperature for a short period before it was cooked, instead of being cooked immediately after removal from —20° F. storage. The above workers found that much of the typical off-odors and flavors were lost at the higher temperature of holding before cooking, possibly by reversal of the reactions which produced them, or more likely by reaction with some other component of the meat product.

Juiciness scores of the radiated meat were low because the meat was too moist. Moistness increased with degree of radiation.

Cooking method. Higher scores for aroma, color of cut surface and flavor were given patties cooked in the electric test oven and skillet than in the electronic oven. Higher aroma and flavor scores were probably caused by the browned surface (Tables 1, 2, 3). Lower scores for color in the electronically cooked patties were due to pink underdone appearing areas, the occurrence of which was irregular and unpredictable. Pinkish areas were also frequently observed in pan drippings and coagulum of electronically-cooked radiated patties. Morgan (14) reported a tendency for cooked radiated meats to have some pink color due to conversion of denatured globin-nicotin-amide hemichrome to the corresponding hemochrome.

Electronic cooking resulted in higher juiciness scores probably because of the shorter cooking time.

TABLE 3

Mean difference between pairs in palatability scores

Paired mean differences	Palatability scores			
	Aroma	Color	Flavor	Juiciness
Test Oven vs. Skillet	0.3	0.0	0.2	−0.2
Skillet vs. Electronic Oven	0.5*	0.7*	0.5*	0.1*
Control vs. 3×10^6 rep lot	3.7**	0.8*	4.0**	0.8**
Control vs. 6×10^6 rep lot	4.2**	1.1**	4.8**	1.1**
Control vs. 6×10^6 rep lot with MSG	4.3**	0.9*	5.1**	1.2**

* Significant, at 5% level.
** Significant, at 1% level.

Significant interaction between pretreatment and cooking method occurred. Scores for aroma, color of cut surface and flavor of control patties were highest when cooked electronically. Perhaps the reason conventional cooking resulted in higher scores for aroma and flavor of radiated meat was because the longer cooking allowed for loss of some of the radiated odor and flavor.

Order of preference. Average order of preference was: first, control; second, 3×10^6 rep lot; third, 6×10^6 rep lot; and fourth, 6×10^6 rep lot containing MSG and incorporated air. The control lot was ranked first by all of the judges; the 3×10^6 rep second by 8 and fourth by 1 judge; the 6×10^6 rep was ranked second by 1, third by 5 and fourth by 3 judges; the 6×10^6 rep lot with MSG and incorporated air was ranked third by 4 and fourth by 5 judges. No one consistently ranked either of the 6×10^6 rep lots better than the other.

All quality factors of radiated meat might have been scored higher, if in addition to precautions already mentioned, the control had not been presented at the same time and the meat had been scored as part of a meal. McGary *et al. (11)* reported that human subjects on a diet containing 35, 65 and 80% of the calories did not detect differences when foods radiated with 3×10^6 rep were added or dropped from the diet. Urbain *(26)* stated that radiated flavor in foods while detected by an expert taster goes unnoticed by a non-expert panel.

Objective Tests

Radiation. *Raw meat:* No significant difference was found in mean a/b ratio of the center cut surface of raw meat due to radiation (Tables 4, 5).

Radiation apparently increased fat content but decreased moisture content of the raw meat (Tables 4, 5). This was noticeable even though the fat and moisture content of the raw ground meat in each lot varied somewhat on each of the 12 cooking days—the fat less than 1% and the moisture content about 1%. As the fat increased the moisture decreased. This range in fat content, and subsequently moisture content, can be expected since it is practically impossible to get fat uniformly distributed in ground meat. In spite of this lack of uniformity in the raw meat of the same lot, radiation apparently effected the content of fat and moisture. The differences in fat and moisture content of the control lot and the 3 lots of irradiated meat were appreciably greater than the differences in the same lot on the 12 test days: Differences (apparent increases) in the mean fat content of the control and the 3×10^6 rep lot ranged from 2.2 to 3.0%, the 6×10^6 rep lot from 4.5 to 5.6% and the 6×10^6 rep + MSG lot from 5.1 to 6.8%.

Differences (apparent decreases) in mean moisture content of the control and the 3×10^6 rep lot ranged from 2.3 to 4.0%, the 6×10^6 rep lot from 4.7 to 4.9% and the 6×10^6 + MSG lot from 5.8 to 7.7%.

Peroxide values increased with radiation, with each degree of radiation, and with the incorporation of MSG and air (Tables 4, 5). Other workers have found increases in peroxide values particularly in the presence of oxygen *(17, 21)*.

Radiation decreased thiamine content to 54.3% in the 3×10^6 rep lot, to 36.9% in the 6×10^6 rep lot and to 26.9% in the 6×10^6 rep + MSG.

TABLE 4

Mean values for objective color, fat, peroxide, moisture and thiamine content of irradiated raw and cooked ground pork [1]

Objective measurements	Pretreatment	Raw	Cooked test oven	Raw	Cooked skillet	Raw	Cooked Electronic Oven	Pretreatment means	
								Raw	Cooked
Color a/b value	Control	1.09	0.51	0.80	0.41	0.84	0.55	0.91	0.49
	3×10^6 rep	1.02	0.41	0.76	0.40	0.85	0.48	0.88	0.43
	6×10^6 rep	0.99	0.41	0.71	0.39	0.87	0.55	0.86	0.45
	6×10^6 rep + MSG	1.22	0.35	0.71	0.40	0.78	0.49	0.87	0.41
	Means	1.06	0.42	0.75	0.40	0.84	0.52		
Percent fat	Control	26.4	24.8	25.5	24.3	25.6	22.4	25.8	23.8
	3×10^6 rep	28.6	24.5	28.4	26.3	28.6	23.8	28.6	24.9
	6×10^6 rep	30.9	26.0	30.6	24.2	31.2	25.4	30.9	25.2
	6×10^6 rep + MSG	31.5	29.1	32.3	28.0	31.9	27.6	31.9	28.2
	Means	29.3	26.1	29.2	25.7	29.3	24.8		
Peroxide value	Control	19.8	35.8	2.2	20.5	4.0	4.8	8.6	20.3
	3×10^6 rep	56.8	64.8	60.5	81.0	27.8	20.8	48.3	55.5
	6×10^6 rep	46.0	52.8	72.8	90.5	32.5	35.5	50.4	59.6
	6×10^6 rep + MSG	65.5	51.8	87.8	93.8	31.8	29.0	61.7	58.2
	Means	47.0	51.2	55.8	71.4	24.0	22.5		
Percent moisture	Control	58.0	49.6	56.8	48.8	57.8	53.3	57.5	50.6
	3×10^6 rep	54.0	51.5	54.5	49.4	55.0	54.4	54.5	51.8
	6×10^6 rep	53.1	51.2	52.1	50.1	53.1	53.3	52.8	51.5
	6×10^6 rep + MSG	51.5	48.1	51.1	49.6	52.0	51.3	51.6	49.7
	Means	54.2	50.1	53.6	49.5	54.5	53.1		
Thiamine mg./100 g. moist, fat basis	Control	0.555	0.702	0.661	0.715	0.643	0.676	0.620	0.698
	3×10^6 rep	0.329	0.324	0.344	0.342	0.331	0.379	0.335	0.348
	6×10^6 rep	0.240	0.181	0.226	0.177	0.214	0.239	0.227	0.199
	6×10^6 rep + MSG	0.159	0.120	0.164	0.141	0.175	0.182	0.166	0.148
	Means	0.321	0.332	0.349	0.341	0.341	0.369		
dry, fat-free basis	Control	3.37	2.74	4.07	2.66	3.65	2.80	3.70	2.73
	3×10^6 rep	2.94	1.36	1.89	1.41	2.06	1.74	2.30	1.50
	6×10^6 rep	1.45	0.79	1.32	0.70	1.44	1.13	1.40	0.87
	6×10^6 rep + MSG	0.99	0.54	1.05	0.63	1.09	0.86	1.04	0.68
	Means	2.19	1.36	2.08	1.35	2.06	1.63		

[1] Patties weighing approx. 100 g., 3 in. dia. and ½ in. thickness were cooked in the 2 ovens to 190° F. and in skillet to 170° F.

TABLE 5

Mean squares and significance of objective color, fat, peroxide, moisture and thiamine content of raw and cooked ground pork

Source of variance	d. f.	Mean Squares								
		Color a/b ratio		Percent fat		Peroxide value		Percent moisture		Thiamine [1]
		Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Cooked
Pretreatment	3	68	136	86.79**	42.70**	6434**	4234**	81.06**	11.05	10.3004
3×10^6 vs. 6×10^6	1	30	24	31.97**	0.57	26	100	18.20**	0.30	2.3751**
$3 \times 10^6 + 6 \times 10^6$ vs. 6×10^6 + MSG	1	0	76	38.87**	81.28**	1209	3	34.31**	31.34**	2.0978**
Control vs. all irradiated	1	173	309	189.52**	46.24**	18068**	12600**	190.67**	1.52	26.4282**
Cooking Methods	2	4060**	618**	0.13	7.07*	4317*	9677**	3.31	59.70**	0.4083
Test oven vs. Electronic oven	1	3894**	741**	0.00	13.39*	4232*	6613*	0.91	71.10**	0.5913**
Skillet vs. Electronic oven and Test oven	1	4227**	495*	0.26	0.75	4401*	12742**	5.70*	48.31**	0.2252*
Cooking Method × Pretreatment	6	116*	48	0.62	4.08	733	884	0.68	3.24	0.0297
Test oven vs. Electronic oven × 3×10^6 vs. 6×10^6	1	33	36	0.05	0.02	240	716	0.72	0.77	0.0018
Test oven vs. Electronic oven × $3 \times 10^6 + 6 \times 10^6$ vs. 6×10^6 + MSG	1	527**	21	0.09	1.02	208	83	0.01	0.68	0.0021
Test oven vs. Electronic oven × Control vs. all irradiated	1	27	92	1.50	3.41	140	14	0.84	1.28	0.1225
Skillet vs. Ovens × 3×10^6 vs. 6×10^6	1	23	27	0.08	18.50*	310	88	1.40	2.85	0.0196
Skillet vs. Ovens × $3 \times 10^6 + 6 \times 10^6$ vs. 6×10^6 + MSG	1	35	32	1.54	0.90	313	218	0.03	13.02**	0.0270
Skillet vs. Ovens × Control vs. all irradiated	1	51	82	0.48	0.63	3187	4186*	1.10	0.83	0.0055

[1] Dry, fat-free basis.
* Significant at 5% level.
** Significant at 1% level.

Mean retention in the 3×10^6 rep lot was appreciably higher than that reported by Groninger *et al. (7)* in ground pork which was thawed and irradiated by gamma rays and then refrozen. Irradiation in the frozen state in the study herein reported might have been protective of thiamine. Oxygen, which was present, is probably not responsible for thiamine destruction. Several workers have reported decrease in thiamine content during radiation *(1, 6, 12)*.

The pH remained fairly constant in the raw meat, 6.2 to 6.4.

Cooked meat: The objective findings with the raw meat carried over to the cooked meat with two exceptions. The 6×10^6 rep lot + MSG contained the least moisture, possibly because these patties flattened out during cooking. Differences were slight. Differences in thiamine content of the two 6×10^6 rep lots were not significant. This may also have been due to the MSG lot requiring shorter cooking.

The pH remained fairly constant, 6.4 to 6.5.

Cooking method. Electronic cooking resulted in an apparent lower fat content, less increase in peroxides and higher moisture and thiamine content than did the electric test oven or skillet cooking (Tables 4, 5, 6). It may be that in electronic cooking more fat was lost to the drip. Drip in this study was not analyzed but Thomas *et al. (24)* found that drip of electronically-cooked unirradiated pork patties was half fat and half water. It has been reported that microwaves penetrate fat more rapidly than lean. Thus more fat may have been rendered from electronically-cooked than conventionally-cooked meat patties.

The mean fat content of the electronically-cooked patties was 24.8% which was slightly less than that of the patties cooked by conventional methods, 26.1 and 25.7%.

The lower peroxide content and the higher moisture and thiamine content may be due to shorter cooking time in the electronic oven, about one-third, than in the two conventional methods. No doubt covering of the pan in electronic cooking was a factor in the higher moisture content. Peroxide values did not increase during the short electronic cooking as they did in conventional cooking.

Retention of thiamine in electronic cooking. During electronic cooking, retention of thiamine in the control lot was less, 70.1%, than in the radiated lots, 77.3%, 74.0% and 80.5% (Table 7). The control lot required the longest cooking time, and the 6×10^6 rep + MSG, with incorporated air, lot the shortest, to reach the same internal temperature. This was probably, at least partially, because the control patties increased in thickness and became more dense during cooking and the MSG lot decreased in thickness and became loose in texture. Causey *et al. (3)* reported a higher mean thiamine retention, 85%, during electronic cooking of unirradiated frozen pork patties. These workers found a considerable amount of thiamine in the drip and suggested that little, if any, was destroyed by the short cooking. The drip in this study was not analyzed.

The greater destruction of thiamine with short increase in cooking time can be expected in view of the fact that studies in this and other laboratories have shown about the same thiamine content in meat cooked conventionally and electronically even though the former may have required 3 to 4 times as long.

TABLE 6

Mean differences between pairs [1] in objective color, fat, peroxide, moisture and thiamine content of raw and cooked irradiated ground pork

Paired mean differences	Color a/b ratio		Percent fat		Peroxide value		Percent moisture		Thiamine [2]
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Cooked
Test Oven vs. Skillet	31**	2	0.1	0.4	−8.8	−20.2	0.6	0.6	0.01
Skillet vs. Electronic Oven	−9*	−12**	−0.1	0.9	31.8**	48.9**	−0.9*	−3.6**	0.28**
Control vs. 3×10^6 rep	3	6	−2.8**	−1.1	−39.7**	−35.2**	3.0**	−1.2	1.23**
Control vs. 6×10^6 rep	5	4	−5.1**	−1.4	−41.8**	−39.3**	4.7**	−0.9	1.86**
Control vs. 6×10^6 rep + MSG	4	8	−6.1**	−4.4**	−53.1**	−37.9**	5.9**	0.9	2.05**
6×10^6 rep vs. 6×10^6 rep + MSG									0.19†

[1] Dunnett, C. W. A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.*, **50**, 1096-1121 (1955).

[2] Dry, fat-free basis.

* Significant at 5% level.

** Significant at 1% level.

† Almost significant but not quite.

TABLE 7

Effect of irradiation on thiamine retention in raw and electronically cooked ground pork

Radiation dosage	Thiamine Retention		
	Raw	Electronically Cooked	
		Cooking only	Radiation + Cooking
	%	%	
Control	100	70.1	—
3 × 10^6 rep	54.3	77.3	41.7
6 × 10^6 rep	36.9	74.0	26.9
6 × 10 6 rep + MSG	26.9	80.5	21.6

Electronic cooking resulted in less loss of thiamine than did radiation.

Irradiation plus electronic cooking. Mean thiamine retentions during radiation plus electronic cooking for the 3 lots were 41.7%, 26.9% and 21.6% (Table 7).

SUMMARY

A study was made of the effect of beta radiation on the quality of ground pork. Four lots were studied: control, 3 × 10^6 rep, 6 × 10^6 rep, and 6 × 10^6 rep + monosodium glutamate and incorporated air. The ground pork was frozen and kept frozen during shipping, radiation, and storage from 4 to 13 weeks. It was not radiated in the absence of oxygen and was exposed to oxygen after radiation during mixing, reshaping and freezing.

Effects of cooking the four lots of meat from the frozen state in an electronic oven, an electric test oven and in an electric skillet were compared.

Radiation resulted in significantly lower aroma, flavor, color and juiciness palatability scores; in preference; in cooking times; thickness after cooking; and moisture and thiamine content. Radiation resulted in increased diameter of patties during cooking; in apparent fineness of grind; in softness, moistness, waxiness and looseness of texture, and in apparent fat and peroxide content of both raw and cooked meat. Radiation did not significantly effect pH, or a/b color ratio of the cut surface of either raw or cooked meat.

Electronic cooking resulted in higher juiciness scores, evaporation loss, moisture and thiamine content, and a/b color ratio of the cut surface than did the two conventional methods. It resulted in lower aroma, flavor and color scores; cooking drip loss; peroxide and fat content. However, significant interaction between pretreatment and cooking method was observed: control patties received higher aroma, color and flavor scores when cooked electronically than by the 2 conventional methods. On the other hand, aroma, color and flavor scores of the radiated patties were lower for patties cooked electronically than for those cooked conventionally.

In electronic cooking, per cent retentions of thiamine were lowest in the control lot which required the longest cooking, and highest in the 6 × 10^6 rep + MSG lot which required the shortest cooking.

Further studies including radiating and storing the meat in the absence of oxygen, subjecting the control lot to hazards of transportation similar to those of the radiated meat, allowing the radiated meat to stand at room temperature for a period immediately before cooking, and testing it for

palatability as a part of a meal are needed. No doubt higher quality irradiated meat could be obtained with certain precautions.

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EFFECT OF STORAGE ON THE CARBOHYDRATE CONTENT OF TWO VARIETIES OF POTATOES GROWN IN CANADA AND TREATED WITH GAMMA RADIATION [a]

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The sprouting of potatoes may be inhibited by ionizing radiations with doses as low as 3.5 krep., (3.3 krad.), *(2)*. However, there seemed to be some discrepancies in the published results concerning the effect of ionizing radiations on the carbohydrate content of stored potatoes. Sparrow and Christensen *(3, 8)* observed a definite sweetening, but only in potatoes treated at doses higher than 20 krep., (18.6 krad.). The same observation was given by Brownell *et al. (1, 9)*. However, Burton and Hannan *(2)* reported that after a minimum of six months of storage at a temperature of 10° C, sweetening rendered tubers irradiated at 8.5 krep., (7.9 krad.), unsuitable for crisp or chip production. Further, Schwimmer *et al. (5, 6)* found that the sugar content (especially sucrose) of irradiated tubers held at a low temperature rose steadily above control levels during the first two weeks after irradiation, while post irradiation storage at a higher temperature resulted in a much more transient increase. Similarly, Sereno *et al. (7)* observed an increase in reducing and total sugars content of irradiated Katahdin potatoes after one year of storage at temperatures of 38° F. and 60° F. The increase was more pronounced at the lower storage temperature.

The effect of radiation on the carbohydrate content of potatoes seemed to depend on the dose of irradiation, the pre- and post-irradiation history of the tubers, and the variety of potatoes studied. In view of this phenomenon, it was considered of value to study the long term effect of gamma radiation on the carbohydrate content of two varieties of potatoes grown commercially in Canada: the Katahdin and Netted Gem (Russet Burbank) varieties.

MATERIAL AND METHOD

The potatoes were grown in 1956 under identical conditions. Twelve hundred tubers from each of the 2 varieties were sorted randomly into 4 treatment groups: control, 7.9 krad., 10.2 krad. and 14.0 krad. Those selected for irradiation were exposed for predetermined periods to gamma radiation from spent fuel rods removed from the N.R.X. reactor, Chalk-River, Ontario. The maximum variation in dosage due to sample volume was estimated to be of the order of 25% using silver glass dosimeters. Following

[a] This work forms part of a cooperative project set up in 1956 between the Canada Department of Agriculture, the Atomic Energy Company of Canada, and the Department of National Health and Welfare to study the effect of gamma radiation on the storage and culinary properties, ascorbic acid, reducing sugars, sucrose and starch content of two commercially important varieties of potatoes grown in Canada: the Katahdin and Netted Gem (Russet Burbank). The experimental design and results of this study were presented at the 15th International Horticultural Congress in Nice, France, April 1958. Acknowledgement is made to the committee in charge of this project for permission to publish these results.

irradiation, half of the tubers of each treatment were then stored at 40° F. and the other half at 68° F. The relative humidity was maintained at 80%. At the end of one week, 4½ months and 9 months after irradiation, 5 tubers from each treatment group were randomly selected for analysis of reducing sugars, sucrose and starch. The first 2 series of analyses were made on individual tubers but 2 composite samples each containing 2½ tubers were used for the final series of tests. This procedural change was justified by the small variation observed between tubers in the first 2 sets of data. At 4½ months and 9 months of storage, a further group of 5 Netted Gem tubers randomly selected from each treatment at 40° F. were "reconditioned", i.e. they were placed in storage at 70° F. for an additional 3 weeks, then analyzed. Duplicate determinations were carried out whenever required.

For analysis each tuber was cut and immediately passed through a meat grinder. Aliquots of 25 g. were prepared in 80% alcohol according to A.O.A.C. method *(4)* and stored at 40° F. until analysis. Storage under these conditions for periods of up to 2 months had no deleterious effect on the stability of the preparations. The reducing sugars were determined using the Munson-Walker general method, and the amount of reduced copper was obtained by direct weighing of cuprous oxide. Sucrose was calculated from reducing sugars before and after inversion. Starch was analyzed by the direct acid hydrolysis method.

RESULTS

Results for reducing sugars, sucrose, and starch content determinations are given in Tables 1, 2 and 3. No data are presented for the control treatments of both varieties after 9 months of storage at 68° F. since these had already been discarded on account of decay. Table 4 summarizes the analysis of variance for all the data. Because of the possibility of heterogeneous response to storage between the 2 storage temperatures, both the orthogonal and hierarchical division of the total variation was performed despite uncertainties as to the probability level in the significance tests for the latter.

Reducing sugars. At the end of the first week following irradiation, the irradiated tubers of both varieties had a higher content of reducing sugars than those of the controls; however, after 4½ months of storage at 40° F., these differences had disappeared following a general increase. During the same storage interval, highly significant differences between control and radiation treatments for both varieties persisted at the 68° F. storage temperature, the reducing sugars values for the control treatments being less by about 50%. Over the next 4½ months, a decrease in the content of reducing sugars was observed for the two varieties at both storage temperatures, the reduction for the Katahdin variety being significantly smaller than for the Netted Gem which consistently showed lower values throughout the period of this study. The irradiated Netted Gem tubers reconditioned normally at the end of 4½ and 9 months of storage at 40° F., i.e. they showed a highly significant decrease in the amount of reducing sugars present after a three week storage at 70° F. following the above storage periods.

Sucrose. One week after irradiation, all radiation treatments yielded significantly higher sucrose content values than the control treatments. At the 40° F. storage temperature, the sucrose content of the Katahdin variety increased gradually during the 9½ months of storage. The opposite effect was observed for the Netted Gem variety. However, the control and radiation treatments of both varieties followed the same general pattern and gave about the same mean sucrose values. During the first 4½ months of storage at 68° F., the sucrose content of both varieties increased; however, the sucrose content of the control treatment remained significantly lower than that of any of the radiation treatments. Over the next 4½ months at the same storage temperature, a fourfold increase in the sucrose content of all the radiation treatments was recorded for both varieties. Unfortunately, no data for the control treatments were available because they had already been discarded on account of decay.

There were significant differences between treatments throughout the 9 months of storage; however, our experimental results do not seem to favour persistently any given treatment for both varieties. The Netted Gem variety, except for the determinations one week after irradiation, yielded values which were significantly lower than the corresponding ones for the Katahdin variety. Reconditioning of the Netted Gem tubers produced an effective decrease in the sucrose content of the radiation treatments after 4½ months of storage and an increase for the same treatments after 9 months of storage. The opposite effects were observed when reconditioning the control treatments.

TABLE 1

Effect of storage on the content of reducing sugars of potatoes after treatment at different levels of gamma radiation

Storage		No. of replicates analyzed	Mean Reducing Sugars (% fresh weight) for treatments			
Months	Temperature		Control	7.9 krad.	10.2 krad.	14.0 krad.
Katahdin Variety	(°F.)					
¼	52-54	5	0.325	0.729	0.516	0.504
4½	40	5	1.26	1.26	1.26	1.23
9	40	2	1.12	1.09	1.25	1.38
4½	68	5	0.162	0.384	0.309	0.425
9	68	2	discarded	0.274	0.304	0.392
Netted Gem Variety						
¼	52-54	5	0.274	0.441	0.373	0.535
4½	40	5	1.08	0.955	0.907	0.957
4½	Recond.*	5	0.396	0.119	0.082	0.210
9	40	2	0.776	0.485	0.579	0.496
9	Recond.*	2	0.266	0.281	0.242	0.295
4½	68	5	0.255	0.502	0.470	0.524
9	68	2	discarded	0.265	0.325	0.303

* Reconditioned: potatoes removed from 40° F. storage to 68° F. for 3 weeks.

TABLE 2

Effect of storage on the sucrose content of potatoes after treatment at different levels of gamma radiation

Storage		No. of replicates analyzed	Mean Sucrose (% fresh weight) for treatments			
Months	Temperature		Control	7.9 krad.	10.2 krad.	14.0 krad.
Katahdin Variety	(°F.)					
¼	52-54	5	0.128	0.492	0.358	0.342
4½	40	5	0.372	0.396	0.557	0.583
9	40	2	0.703	1.01	0.775	0.565
4½	68	5	0.389	0.785	0.748	0.815
9	68	2	discarded	2.86	3.08	2.75
Netted Gem Variety						
¼	52-54	5	0.154	0.502	0.363	0.349
4½	40	5	0.213	0.235	0.274	0.261
4½	Recond.*	5	0.231	0.089	0.092	0.283
9	40	2	0.194	0.186	0.244	0.159
9	Recond.*	2	0.110	0.284	0.260	0.304
4½	68	5	0.405	0.549	0.666	0.617
9	68	2	discarded	2.41	2.36	2.74

* Reconditioned: potatoes removed from 40° F. storage to 68° F. for 3 weeks.

TABLE 3

Effect of storage on the starch content of potatoes after treatment at different levels of gamma radiation

Storage		No. of replicates analyzed	Mean Starch (% fresh weight) for treatments			
Months	Temperature		Control	7.9 krad.	10.2 krad.	14.0 krad.
Katahdin Variety	(°F.)					
¼	52-54	5	13.95	13.83	13.48	14.05
4½	40	5	11.49	12.02	11.64	11.20
9	40	2	12.46	12.65	11.53	11.77
4½	68	5	16.01	11.96	13.04	12.47
9	68	2	discarded	11.12	9.63	10.02
Netted Gem Variety						
¼	52-54	5	15.46	14.26	15.89	14.94
4½	40	5	15.24	15.75	14.05	14.50
4½	Recond.*	5	15.25	15.94	14.45	14.63
9	40	2	14.14	15.29	15.01	14.42
9	Recond.*	2	14.72	15.26	14.30	14.63
4½	68	5	17.81	14.64	14.22	15.12
9	68	2	discarded	12.38	13.28	13.19

* Reconditioned: potatoes removed from 40° F. storage to 68° F. for 3 weeks.

TABLE 4

Summary of significance tests in analysis of variance and standard deviations

Source of Variation	Reducing Sugars			Sucrose			Starch		
	¼	4½ months	9	¼	4½ months	9	¼	4½ months	9
a) Orthogonal									
Between Treatments	**	.....	.....	**	**	**	.....	*	.....
Between Varieties	*	**	*	.....	**	**	*	**	**
Treat. × Time	.....	.....	.....	.....	.....	.....	.....	.....	.....
Between Temp. within Treat. and Var.	.....	**	**	.....	**	**	.....	.....	*
Standard Deviation	0.71	0.66	0.34	0.39	0.83	0.29	1.22	1.48	0.84
b) Hierarchical									
Between Treatments	***	.....	.....	***	**	**	***	*	.....
Between Temp. within Treatments	***	**	.....	***	**	**	***	.....	.....
Between Var. within Temp. within Treat.	***	**	**	***	**	**	***	**	**
Standard Deviation	***	0.66	0.34	***	0.83	0.29	***	1.48	0.84
c) Combined Analysis (all data)									
Between Storage Times		.....			**			**	
Between Treatments within Time		.....			**			.....	
Between Varieties within Time		**			**			**	
T × V within Time		.....			.....			.....	
Standard Deviation		1.44			1.52			1.46	

* Significant at P = .05.
** Significant at P = .01.
*** Not applicable.

Starch. The starch content of all treatments of both varieties dropped to a relatively constant value after 4½ months of storage at 40° F. However, at 68° F., while the radiation treatments of both varieties showed a regular decrease throughout the 9 months of storage, the control treatment of the 2 varieties yielded a significant increase after 4½ months of storage. Thus, at the end of 4½ months of storage at 68° F., the control treatments of both varieties had significantly higher mean starch content values than any of the radiation treatments. Reconditioning had no significant effect on the starch content of the Netted Gem potatoes.

DISCUSSION

The immediate effect of gamma radiation on the 2 varieties of potatoes studied was an increase in reducing sugars and sucrose. However, low temperature storage (40° F.) restored the initial condition, i.e. the reducing sugars and sucrose contents of the treated potatoes after 4½ months and 9 months of storage compared favourably with those of the control potatoes. Further, the irradiated potatoes of the Netted Gem variety reconditioned normally at the end of the above two storage periods.

At a higher storage temperature (68° F.), where sprouting of untreated potatoes soon becomes a serious problem and where some kind of sprouting inhibitor is required, there seemed to be some detrimental effects, as follows. After 4½ months of storage at 68° F., the reducing sugars and sucrose contents of the treated potatoes of both varieties were twice as large as the corresponding control treatments which were, as could be expected, significantly higher in starch content. Over the next 4½ months of storage, the control treatments were lost on account of decay. However, the concentation of sucrose in the preserved potatoes of both varieties increased fourfold and a corresponding decrease in starch content was observed. Thus it appears that room temperature storage of potatoes, although highly practical and economic, is not an ideal storage temperature for any long period of time even if sprouting of the potatoes can easily be eliminated with various types of radiations or delayed with some known chemical agents. The reason is that the normal enzymatic processes, which are mainly temperature dependent, are still present.

The above results from the study of 2 commercially important varieties of potatoes grown in Canada, are in line with the published results concerning the same 2 varieties and other varieties grown in other countries. However, the increase in the sucrose content was observed at doses lower than 20 krep., (18.6 krad.), contrary to the findings of Sparrow and Christensen *(3, 8)*, and at a temperature higher than those given by Schwimmer *et al. (5, 6)* and Sereno *et al. (7)*. The 7.9 krad. treatment effected 100% sprout control, as reported by Burton and Hannan *(2)*; further, there was no significant difference between the effect of the selected 3 irradiation levels on the carbohydrate content of these 2 varieties of potatoes.

SUMMARY

A study of the effect of gamma radiation on the carbohydrate content of two commercially important varieties of potatoes grown in Canada, the Katahdin and Netted Gem (Russet Burbank), was performed. The tubers were given three doses of gamma radiation, 7.9 krad., 10.2 krad. and 14.0 krad., and then held for 9 months at two storage temperatures, 40° F. and 68° F. No significant difference was observed between the effect of the

three doses. The irradiated tubers showed an initial rise in reducing sugars and sucrose contents which was counter-balanced after 4½ months of storage at 40° F. Gamma irradiation made possible prolonged storage at room temperature; however, a large increase in sucrose content was observed in the treated tubers after 9 months of storage at 68° F.

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BEHAVIOR OF STARCH DURING FOOD PREPARATION. II. EFFECTS OF DIFFERENT SUGARS ON THE VISCOSITY AND GEL STRENGTH OF STARCH PASTES [a, b]

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The properties of food products thickened with starch are influenced to varying degrees by the other ingredients present. Sucrose has been reported *(3, 15, 17)* to inhibit the swelling of starch granules with resulting increase in the temperature of the initial rise in hot-paste viscosity, lowered maximum viscosity, decreased tendency of the paste to thin after reaching maximum viscosity, decreased diffusion of soluble starch from the swollen granules, and decreased strength of the gels formed from the cooled paste. Starch-conversion sirup solids are also known to inhibit pasting and gelling of starch *(1)*. The tender gel structure of puddings made with whey in place of or in addition to nonfat milk solids has been attributed to the increased concentration of lactose as well as to the decreased protein content *(2)*. Hanning, Bloch, and Siemers *(2)* reported that corn starch puddings made with nonfat milk solids and with the same weight of sucrose, glucose or lactose were equal in gel strength when measured by a penetrometer test devised by them.

A more complete comparison of the effects of different sugars at various concentrations on the viscosity and gel strength of starch pastes in systems free of the influence of other ingredients appeared to be essential to a better understanding of the characteristics of sweetened starch-thickened foods. The sugars and sirups used in this study were sucrose, dextrose, fructose, maltose, lactose, an invert sirup, and corn sirups of three different levels of hydrolytic conversion. Sorbitol was also included in the study because of its structural relationship to the sugars and because of its use in foods, especially as a sweetening agent in the diets of diabetic patients *(12)*.

EXPERIMENTAL METHODS

Materials. The starch used in this study was a sample of ordinary commercially prepared corn starch. Sugars used were C.P. Special D-fructose, C.P. Special D-lactose monohydrate, C.P. D-maltose monohydrate, commercial anhydrous dextrose (Cerelose), and commercial sucrose. The invert sugar sirup was a commercial sample, 55% inverted. Corn sirups were commercial samples of C.S.U. of 64 D.E., 56 D.E. and 43 D.E., which had the following content *(13)* of glucose, maltose, and trioses and above, respectively: 64 D.E., 37, 30, 33; 56 D.E., 32, 18, 50; 43 D.E., 20, 15, 65. The first was an acid-enzyme converted product; the last two were acid converted. The D-sorbitol was a pure crystalline product.

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The basic mixture was a 5.0% corn starch paste, calculated on the dry weight basis. Account was made for the moisture of the starch, 10.93%, which had been determined by drying in a vacuum oven at 120° C. to constant weight. Sufficient 0.1 *N* sodium hydroxide was added to the starch slurry to produce a cooked paste with a pH value of 6.6.

Dry weights of sugars and sirups used were 5, 10, 20, 30 and 50% of the total weight of water present in the final mixture. Solution of the sugars in part of the water before addition to the starch eliminated any effects of the rates of solution of the various sugars on the gelatinization of the starch. Sirups were diluted to a relatively low viscosity to facilitate mixing.

Hot-paste viscosity. Uniform cooking and a continuous record of hot-paste viscosity during cooking were obtained by use of a Corn Industries Viscometer, described by Kesler and Bechtel *(7)*. Heating and gelatinization of the starch mixture in the stainless steel beaker was accomplished with a glycerol-water bath at 100° C., rather than the more commonly employed water bath at 92° C., to produce more complete gelatinization of the starch and to approximate more closely the conditions normally used during food preparation.

Cooking was terminated 5 minutes after the maximum viscosity was reached. When a decrease in viscosity failed to occur, the cooking was arbitrarily stopped 5 minutes after the viscosity appeared to be constant.

Gel strength. A modification of the Saare and Martens embedded disk method *(11)* employing a recording gel tester similar to that described by Hjermstad *(4)* was used. The containers used were 250-ml. Griffin Pyrex beakers. The samples were refrigerated for 18 hours at about 3° C. and allowed to stand in room atmosphere for 15 minutes before testing for strength. Although the gel strength of samples was found to decrease rapidly during the first 10 minutes after removal from the refrigerator, changes thereafter were very slow and did not introduce any appreciable error into the values obtained.

Readings with the Corn Industries Viscometer were not completely reproducible for either viscosity or gel strength. However, control samples tested within a single day showed little variation. Therefore, all concentrations of a single sugar were studied in a single day in random order, with control samples before and after the series containing sugar. Duplicate experiments were carried out on each sugar at each concentration.

RESULTS AND DISCUSSION

Qualitative effects of different sugars and sirups on hot-paste viscosity were very similar (Table 1). Lower concentrations of sugars tended to increase the maximum hot-paste viscosities, while amounts greater than 20% of the weight of the water present progressively decreased the viscosities. An analysis of variance of these data showed that differences in the maximum

TABLE 1

Effect of sugars on maximum hot-paste viscosity [1] of corn starch

Sugar	Concentration of sugar					
	0	5%	10%	20%	30%	50%
Fructose	118	133	142	150	147	134
Glucose	115	136	142	148	147	127
Sorbitol	115	131	137	141	133	107
Maltose	114	128	139	136	133	95 [2]
Lactose	118	131	132	135	133	92 [2]
Sucrose	124	132	137	141	132	109
Invert sirup	120	132	138	143	140	119
C.S.U. 64 D.E.	116	134	134	140	129	100 [2]
C.S.U. 56 D.E.	116	124	131	133	127	88 [2]
C.S.U. 43 D.E.	119	126	133	127	120	82 [2]

[1] g.-cm. (average of 2 experiments).
[2] No maximum.

viscosity due to the type of sugar present, although not significant at the lower concentrations, were highly significant at the 20, 30 and 50% levels.

In addition to affecting the maximum hot-paste viscosities attained, increasing concentrations of sugars and sirups were found to change the shapes of the gelatinization curves. Those obtained with sucrose were typical (Figure 1). The highest maximum viscosities were usually obtained with sugar concentrations of about 20%. The heating period required to produce sufficient increase in viscosity to be recorded by the viscometer was not appreciably changed by small concentrations of sugar, but became slightly longer with 30% concentrations of some sugars and was markedly longer in all cases with 50% concentrations. With the higher sugar concentrations, the increase in viscosity not only was delayed, but, once started, occurred more slowly. Likewise, decrease in viscosity after maximum value had been reached was retarded or, in some cases, prevented.

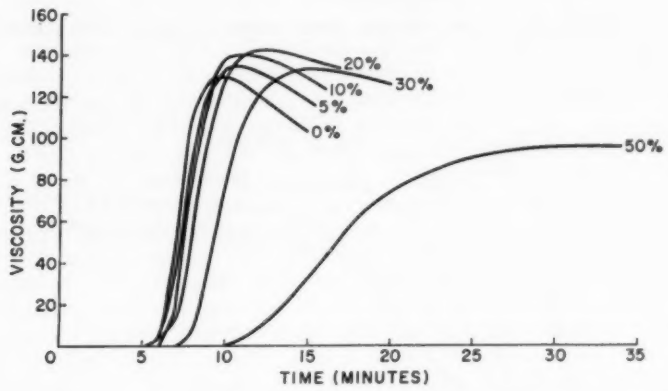

Figure 1. Effect of sucrose on gelatinization of 5% corn starch.

The general shapes of the gelatinization curves were quite similar when different sugars were used at low concentrations. However, differences between the curves obtained with various sugars at the highest concentration were pronounced (Figure 2). All sugars delayed gelatinization. The effects of monosaccharides were not as great as those of sorbitol and the disaccharides, especially lactose and sucrose. The maximum viscosity reached was raised by the presence of monosaccharides but lowered by disaccharides.

The invert sirup, 55% inverted, gave curves lying between the curves for sucrose and those for glucose and fructose at corresponding concentrations. This intermediate position was most striking at the 50% level (Figure 2). Final viscosities of corn starch pastes containing corn sirups of different degrees of conversion also were in the order which might be anticipated from their composition, although the early parts of the curves were not as clearly separated (Figure 3). The 64 D.E. sirup with its higher concentration of dextrose and maltose produced a curve more closely approaching the curves for these individual sugars than did the sirups of lower D.E.

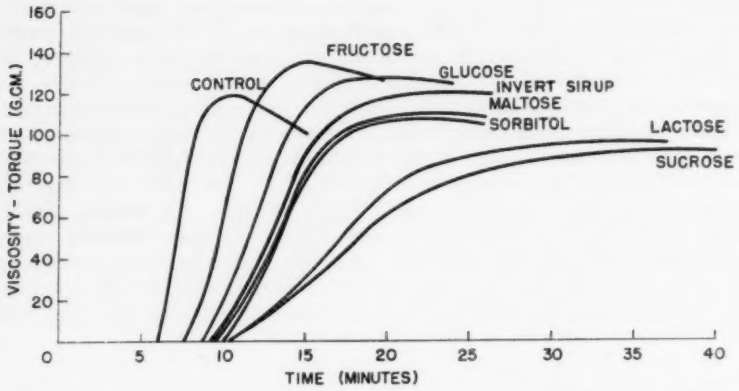

Figure 2. Gelatinization curves for corn starch pastes containing 50% sugars.

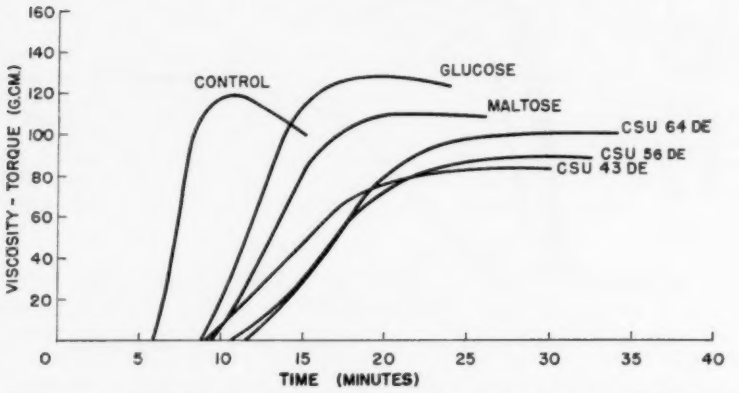

Figure 3. Gelatinization curves for corn starch pastes containing 50% glucose, maltose, or corn sirups.

That the main effect of sugars on starch pastes is inhibition of the swelling of the granules seems apparent from the photomicrographs published by Whittenberger and Nutting *(15)* and Hester, Briant and Personius *(3)*, as well as those in an earlier unpublished thesis of Nevenzel *(9)*. The last of these investigators showed that increasing concentration of sugar caused correspondingly greater depression of the normal swelling of starch granules when heated in water, and that high concentrations greatly increased the temperature at which birefringence disappeared. It was suggested *(9, 15, 17)* that the sugar molecules compete successfully with the starch for the available water, leaving insufficient water for the complete swelling of the granules.

Studies such as that of Taylor and Rowlinson *(14)* tend to support this theory by indications of strong hydrogen bonding between sugars and

the surrounding water molecules. However, sufficient data on the hydration of different sugars in solution do not seem to be available to indicate whether or not differences in their effects on the swelling of starch granules is caused by differences in the extent to which individual sugars can compete with the starch for the available water. That molar concentrations of the sugars or numbers of hydroxyl groups available for hydrogen bonding of water are not the direct explanation for the effects of the various sugars seems apparent from the present study. When solutions of equal concentration by weight were compared the disaccharide solutions with lower molar concentration and fewer free hydroxyl groups caused the greater effects. In quite a different type of system, that of peaches canned in corn sirup, it has been observed *(6)* that the rate of penetration of the members of the maltosaccharide series into fruit is related inversely to the degree of polymerization of the sugar molecule. Whether or not difference in rates of penetration of individual sugars into the starch granules may be a factor in causing differences in their effects on starch gelatinization has not been examined.

Other investigators *(3, 16)* have reported increased viscosities in pastes of starches and flours through addition of small amounts of sucrose. Such an effect appears to be common to all sugars (Table 1). What the explanation of this effect may be is uncertain at present.

Gel strength, determined by the embedded disk method, varied with the concentration and type of sugar or sirup used (Table 2). Although the effect of increasing the concentration was qualitatively similar for all the sugars, there were marked differences between the quantitative effects of the individual sugars. These differences appeared to reflect the differences obtained in the hot-paste viscosity curves. Comparison of Tables 1 and 2 indicated, however, that the hot-paste viscosity maxima are not directly related to the gel strengths. Although a few of the sugars and sirups seemed to increase the gel strength slightly when they were added in a very low concentration, the general effect of all the sugars was a progressive decrease in gel strength with an increase in sugar concentration. At the 50% level of sugar, only fructose and glucose permitted any visible formation of gel structure at all, and their gels were exceedingly tender.

Meyer, Bernfeld, Boissonnas, Gürtler and Noelting *(8)* described a starch gel as a structure formed by the association of the free ends of amylose and amylopectin molecules which had partially diffused from the granule into the surrounding medium, but still had one end anchored in the granule. Since such a structure involves considerable swelling and possible rupture of the granules, pastes exhibiting decreased granular swelling, as shown by a low viscosity curve lacking a maximum, would be expected to produce little, if any, gel strength. Comparison of data for gel strengths (Table 2) and gelatinization curves (Figures 1 and 2) appears to support this theory. Hofstee and de Willigen *(5)* envisioned the structure of a starch gel to be much like that described by Meyer *et al.*, also visualizing the granules as forming an essential part of the net, rather than being embedded as indifferent parts. They observed a resemblance between the viscosigraph curve for potato starch and the curve for gel strengths of samples removed after different cooking times. It was concluded that

TABLE 2

Effect of sugars on gel strength [1] of corn starch

Sugar	Concentration of Sugar					
	0	5%	10%	20%	30%	50%
Fructose	149	157	158	140	96	42
Glucose	146	150	145	104	75	26
Sorbitol	148	161	141	109	74	26 [2]
Maltose	148	143	134	94	66	20 [2]
Lactose	158	157	133	93	60	17 [2]
Sucrose	151	139	127	90	63	15 [2]
Invert sirup	145	152	143	105	70	25 [2]
C.S.U. 64 D.E.	140	145	126	88	52	16 [2]
C.S.U. 56 D.E.	151	135	110	79	48	13 [2]
C.S.U. 43 D.E.	146	135	119	86	54	13 [2]

[1] g.-cm. (average of 2 experiments).
[2] No gel structure apparent.

maximum gel formation was prevented not only by insufficient swelling of the granules during the early stages of cooking, but also by too great disintegration of the granules during prolonged heating. Although this theory has a certain attraction when used in an attempt to explain the results of the present investigation, other studies in this laboratory *(10)* appear to indicate that it is not applicable to many simple starch-water pastes. In spite of the fact that hot-paste viscosity had decreased, gels from pastes of most of the typical food starches cooked 20 minutes after maximum viscosity had been attained in a Corn Industries Viscometer were decidedly stronger than gels from pastes cooked only to maximum viscosity or 5 minutes past maximum viscosity. No theory which will explain all these results seems to have been proposed.

SUMMARY

The effect of ten different sugars and sirups on the hot-paste viscosity curves and gel strength of 5% corn starch paste has been studied. With all the sugars and sirups used, there was a slight increase in the maximum hot-paste viscosity attained during gelatinization with concentrations of sugar up to 10 or 20%, but a decrease occurred with higher concentrations. There were small but consistent differences among the effects of the different sugars. At higher concentrations the disaccharides were more effective in inhibiting gelatinization than were equal concentrations by weight of monosaccharides.

Concentrations of 20% or higher of all sugars and sirups used caused decided decreases in gel strength of the starch pastes.

Acknowledgments

The authors wish to express their gratitude for a grant from the Corn Industries Research Foundation for partial support of this study. They wish to thank the following companies for supplying some of the sugars and sirups used: Corn Products Refining Company, Refined Syrups and Sugars, Inc., and Atlas Powder Company. They also wish to acknowledge the assistance of Dr. William Baten in the statistical analysis of the data.

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EFFECT OF TEMPERATURE DURING THE ROASTING CYCLE ON SELECTED COMPONENTS OF DIFFERENT TYPES OF WHOLE BEAN COFFEE[a]

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(Manuscript received November 8, 1958)

One of the chief concerns of the coffee industry today is to gain a better understanding of what happens to coffee beans when they are roasted. Such knowledge would lead to a more objective approach to coffee roasting, and in turn, give a better insight into how flavor in the roasted beans is developed.

The purpose of the present study was to determine the effect of temperature at specific temperatures during the roasting cycle on selected components of whole bean coffee, namely: total soluble solids, caffeine, trigonelline, chlorogenic acid, and tannin.

EXPERIMENTAL

Material. The whole bean coffee for this study comprised a series of 7 different types; Santos, Maracaibo-Cuban, Medellin-Armenia, Post Crop Colombian, Old Crop Bucaramanga, Peruvian, and Central American, received from The Coffee Brewing Institute, Inc., vacuum-packed in one-pound cans. Each series contained a control or green bean sample, samples taken from the roaster at several specific intervals, and a finished roast sample.

Grinding of samples. The roasted coffee beans were ground in a Waring Coffee Mill set at its finest setting. The beans were passed through the mill several times, the finest particles being removed by sieving each time. The ground samples were then sieved in a Cenco Sieve Shaker for 10 minutes. The sample passing the No. 30 sieve was used for analysis. Owing to the tough nature of the green beans, a Wiley Mill was used instead of the Waring Coffee Mill, with better success. The beans were fed slowly through the mill, using the medium-mesh screen for the first grinding and the fine-mesh screen for the second grinding, after which the ground coffee was sieved in a Cenco Sieve Shaker as for the roasted coffee. The sample passing the No. 30 sieve was used for analysis.

Methods of analyses. Total soluble solids were estimated by the method of Lockhart *(8)*, combined with an adaptation of the official A.O.A.C. method *(1)* to include 5 extractions with hot water and a contact time of 2 minutes for each extraction. Fifteen ml. aliquots of the filtered extracts were used for analysis. It was found necessary to defat the green coffee samples and also those which had been heated to a temperature of less than 375° F. The defatting procedure of Moores, McDermott, and Wood for green coffee *(10)* was used.

Caffeine was estimated by the spectrophotometric method of Ishler, Finucane, and Borker *(4)*; trigonelline by the method of Moores and Greninger *(9)* which provides for chromatographic separation of the trigonelline and spectrophotometric measurement of the purified sample; and chlorogenic acid by the method of Weiss *(16)*. Defatting was not required in the analyses for caffeine and trigonelline. In the case of chlorogenic acid, the green coffee samples, and those which had been heated to a temperature of less than 375° F., were defatted by the procedure of Moores, McDermott, and Wood *(10)* as in the case of the soluble solids.

Tannin was estimated by the official A.O.A.C. method for tannins in tea and coffee *(1)*. Defatting of the green and partially roasted coffee samples was found not necessary.

For calculation of the data to a dry weight basis, moisture was estimated by the method of Schwartzman *(12)*. A drying time of 16 hours was employed.

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[a] Contribution No. 362 from the Department of Food Technology.

[b] Deceased, September 24, 1959.

RESULTS AND DISCUSSION

Data obtained for selected components in the 7 different types of whole bean coffee are presented in Table 1.

TABLE 1

Average values for selected components in whole bean coffee

Type of coffee and constituent analyzed	Values in % dry basis						
	Green bean	Roasted bean at several temperatures in °F					
		300°	325°	350°	375°	400°	Final
Santos [1]							
Total soluble solids	30.4	30.3	28.4	27.5	27.2	—	28.2 (402°)
Caffeine	1.09	1.11	1.14	1.17	1.23	—	1.26 (402°)
Trigonelline	1.15	1.06	0.99	0.97	0.94	—	0.90 (402°)
Chlorogenic acid	6.86	6.67	6.60	6.24	5.96	—	5.25 (402°)
Tannin	2.38	2.26	2.18	2.13	1.98	—	1.81 (402°)
Maracaibo-Cuban							
Total soluble solids	32.7	33.0	29.7	29.4	28.1	29.1	29.3 (419°)
Caffeine	1.17	1.26	1.28	1.33	1.35	1.36	1.39 (419°)
Trigonelline	1.07	1.05	1.03	1.01	0.98	0.91	0.89 (419°)
Chlorogenic acid	7.74	7.52	7.24	6.78	5.91	5.36	4.80 (419°)
Tannin	2.81	2.67	2.55	2.35	2.04	1.89	1.72 (419°)
Medellin-Armenia							
Total soluble solids	31.3	31.0	30.6	29.2	28.5	29.0	30.0 (421°)
Caffeine	1.15	1.25	1.31	1.32	1.35	1.37	1.38 (421°)
Trigonelline	0.97	0.94	0.92	0.91	0.88	0.87	0.86 (421°)
Chlorogenic acid	8.04	7.72	7.38	7.01	6.28	5.35	4.78 (421°)
Tannin	3.04	2.80	2.67	2.58	2.23	1.98	1.80 (421°)
Post-Crop Colombian							
Total soluble solids	31.3	28.5	28.3	27.3	25.8	26.8	29.5 (430°)
Caffeine	1.25	1.23	1.23	1.28	1.31	1.34	1.32 (430°)
Trigonelline	0.96	0.84	0.83	0.82	0.81	0.81	0.73 (430°)
Chlorogenic acid	7.57	6.98	6.72	6.10	5.55	4.79	3.95 (430°)
Tannin	2.91	2.65	2.54	2.25	2.15	1.71	1.40 (430°)
Old-Crop Bucaramanga							
Total soluble solids	30.7	29.3	28.6	27.1	27.1	27.5	29.6 (431°)
Caffeine	1.29	1.30	1.31	1.34	1.39	1.40	1.41 (431°)
Trigonelline	0.93	0.86	0.86	0.86	0.84	0.82	0.78 (431°)
Chlorogenic acid	7.60	7.18	6.97	6.38	5.62	4.86	4.10 (431°)
Tannin	2.98	2.82	2.71	2.52	2.15	1.79	1.54 (431°)
Peruvian							
Total soluble solids	33.7	30.1	29.5	29.0	28.4	29.1	30.6 (413°)
Caffeine	1.19	1.20	1.21	1.21	1.23	1.26	1.29 (413°)
Trigonelline	0.93	0.89	0.88	0.85	0.85	0.83	0.82 (413°)
Chlorogenic acid	8.05	7.65	7.10	6.48	6.24	5.38	5.06 (413°)
Tannin	3.04	2.87	2.65	2.44	2.34	2.08	1.97 (413°)
Central American							
Total soluble solids	34.4	33.1	31.9	31.4	30.0	29.8	30.5 (407°)
Caffeine	1.08	1.10	1.11	1.14	1.22	1.24	1.26 (407°)
Trigonelline	0.96	0.91	0.90	0.89	0.89	0.88	0.88 (407°)
Chlorogenic acid	7.76	7.18	6.66	6.37	6.06	5.42	5.34 (407°)
Tannin	3.01	2.78	2.58	2.44	2.25	2.06	1.99 (407°)

[1] No sample was received for 400° F. temperature due to insufficient time for sampling before completion of the roast.

Values for total soluble solids in the green coffee samples (Table 1) ranged from 30.4 — 34.4%. The highest values were shown by Central American and Peruvian coffee; the lowest by Old-Crop Bucaramangan and Santos types. Values for total soluble solids in the final roast samples (Table 1) ranged fom 28.2 — 30.6%. These findings are in the same general range as those reported by Subrahmanyan *et al. (14)*, for Indian coffee. Little information has appeared in the literature concerning the water soluble solids content of these particular coffee types. Ukers *(15)*, in a comparative study of the composition of coffee from different countries, reported a water soluble solids content for Santos coffee of 31.1% for green coffee and 30.3% for the roasted beans; and for Guatelmalan coffee (a Central American type) of 31.0% for green beans and 30.5% for roasted beans. His findings for these 2 coffee types are within the ranges determined in this study.

Values for caffeine in the green coffee (Table 1) ranged from 1.08 — 1.29%, the highest values being for Old-Crop Bucaramangan and Post-Crop Colombian; the lowest for Santos and Central American. Values for caffeine in the final roast samples (Table 1) ranged from 1.26 — 1.41%, indicating a slightly higher trend in values from roasting. A similar trend was found by Slotta and Neisser *(13)* who studied 14 types of coffee representative of the coffee producing areas of the world. Ishler, Finucane, and Borker *(4)* reported values for caffeine in Santos coffee of 1.03% for green beans and 1.23% for the roasted beans; in Colombian coffee values of 1.14% for green beans and 1.38% for the roasted beans; and in Central American coffee values of 1.15% for green beans and 1.33% for the roasted beans, which are also in agreement with the findings of this study. No essential differences were found, however, by Kogan, DiCarlo, and Maynard *(6)* who studied the effect of roasting on Santos and Colombian coffee.

Values for trigonelline in the green coffee samples (Table I) ranged from 0.93 — 1.15%, the highest values being for Santos and Maracaibo-Cuban coffee; the lowest for Old-Crop Bucaramanga and Peruvian coffee samples. Values for trigonelline in the final roast samples (Table I) ranged from 0.73 — 0.90%, indicating decomposition of this component from roasting. With the exception of Santos coffee, the losses paralleled the temperature; the higher the temperature of the final roast sample, the greater was the loss of trigonelline. Slotta and Neisser *(13)* Hughes and Smith *(2, 3)* and Moores and Greninger *(9)*, reported that the amount of trigonelline lost during roasting was dependent on the temperature of the roast, which agrees with these findings.

Values for chlorogenic acid in the green coffee samples (Table I) ranged from 6.86 — 8.05%; those for tannin ranged from 2.38 — 3.04%. Values for both components were highest for Medellin-Armenia and Peruvian coffee anl lowest for Santos coffee. Values for chlorogenic acid in the final roast samples (Table I) ranged from 3.95 — 5.34%; those for tannin ranged from 1.80 — 1.99%. Losses for both components paralleled each other and were in direct relation to the temperature of the final roast which is in agreement with literature findings *(13, 5, 11, 7)*.

A summary of the data for moisture content throughout the roasting cycle for the 7 different types of whole bean coffee is presented in Table 2. Values for moisture in the green coffee samples ranged from 7.49 — 11.6%,

TABLE 2

Average values for moisture content in whole bean coffee

	% Moisture						
	Green bean	Roasted bean at several temperatures in °F					
		300°	325°	350°	375°	400°	
Santos [1]	8.24	4.39	3.47	2.78	2.33	—	2.60 (402°)
Maracaibo-Cuban	7.49	5.50	4.16	3.36	2.13	1.85	2.09 (419°)
Medellin-Armenia	8.81	6.74	5.55	5.47	2.37	1.74	2.03 (421°)
Post-Crop Colombian	10.7	5.07	3.74	3.22	2.11	1.75	2.02 (430°)
Old-Crop Bucaramanga	9.68	4.72	3.86	3.01	2.22	1.77	2.04 (431°)
Peruvian	11.6	6.25	4.82	3.94	2.99	1.68	2.27 (413°)
Central American	10.5	6.37	4.46	3.63	2.77	1.77	2.57 (407°)

[1] No sample was received for 400° F temperature due to insufficient time for sampling before completion of the roast.

which approximates the range given by Lockhart *(7)* in his composite analysis of green coffee. Values for moisture in the final roast samples (Table 2) ranged from 2.02 — 2.60% which is in agreement with the findings cited by Lockhart *(7)* that "the moisture content of the beans is reduced to about 2 or 3% during roasting." The lowest moistures for all types (Table 2) were found consistently at the 400° F temperature. The final moisture contents were higher than those at 400° F because water is usually sprayed in at the higher temperature to quench or check the roast.

To show the effect of temperature during the roasting cycle on the components of each type of whole bean coffee, the data (Table 1) have been plotted as per cent retention versus temperature in degrees Fahrenheit, and are shown for Santos, Maracaibo-Cuban, Medellin-Armenia, Post-Crop Colombian, Old-Crop Bucaramanga, Peruvian, and Central American types, in Figures 1-7, respectively.

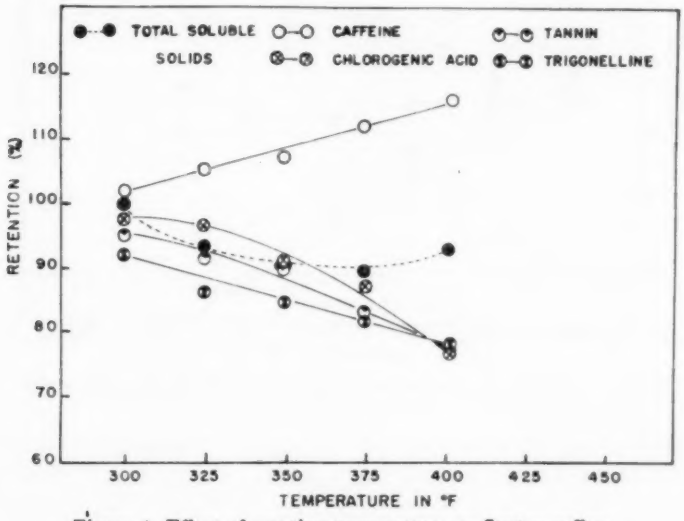

Figure 1. Effect of roasting temperature on Santos coffee.

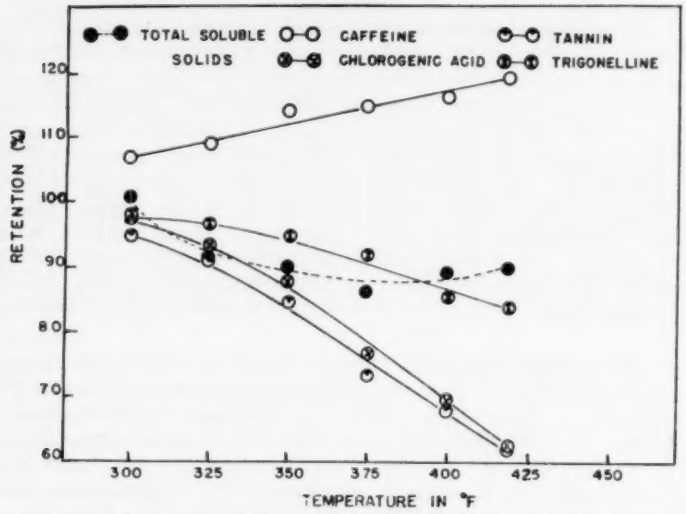

Figure 2. Effect of roasting temperature on Maracaibo-Cuban coffee.

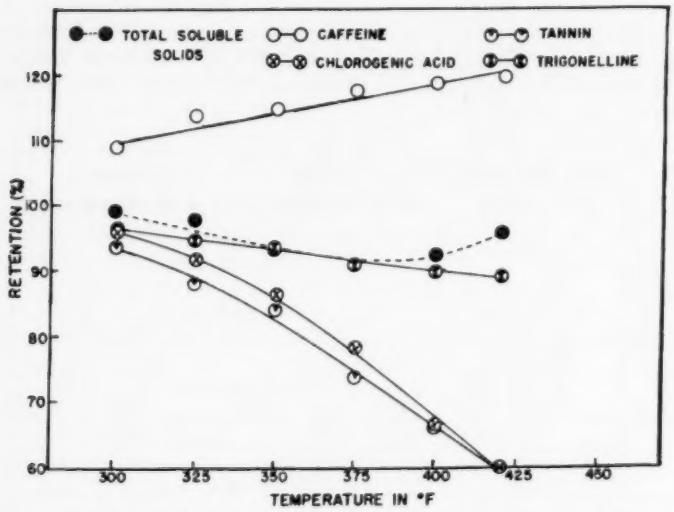

Figure 3. Effect of roasting temperature on Medellin-Armenia coffee.

Total soluble solids (Figures 1-7) decreased very gradually until 375° F. was reached, and then increased with temperature to the end of the roasting cycle, with the exception of the solids for Central American coffee which leveled off at this point.

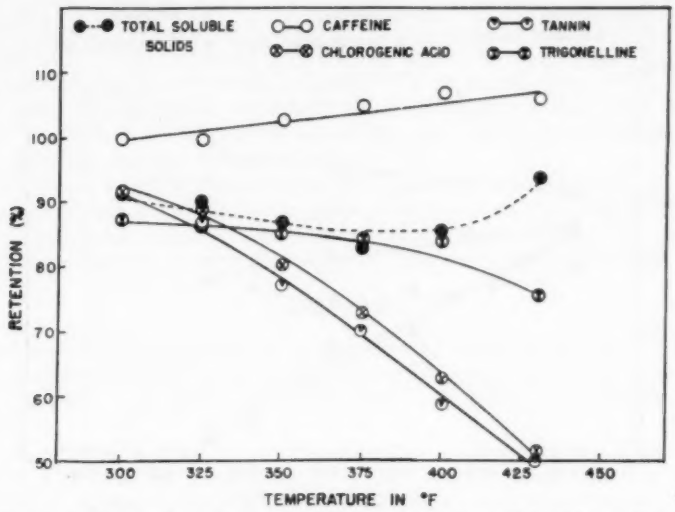

Figure 4. Effect of roasting temperature on Post-Crop Colombian coffee.

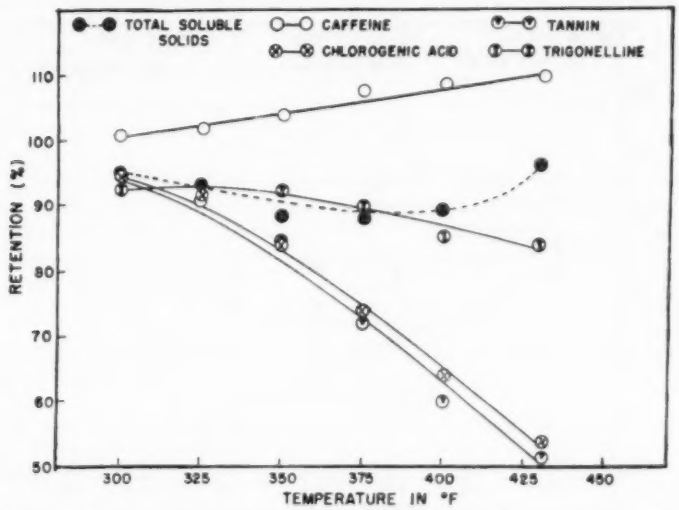

Figure 5. Effect of roasting temperature on Old-Crop Bucaramanga coffee.

Caffeine (Figures 1-7) increased directly with temperature, the increase being gradual for Post-Crop Colombian, Old-Crop Bucaramanga, and Peruvian coffees; more rapid for Santos and Central American coffee types; while for Maracaibo-Cuban and Medellin-Armenia coffee types, the change with temperature was immediate, followed by a gradual increase to the end of the roasting cycle.

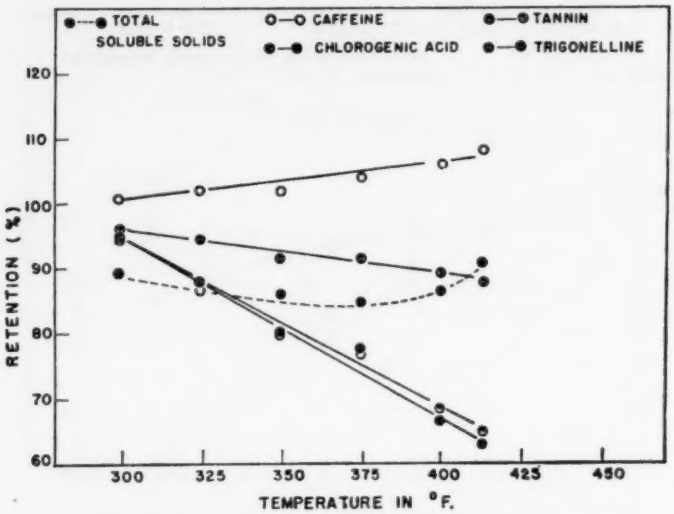

Figure 6. Effect of roasting temperature on Peruvian coffee.

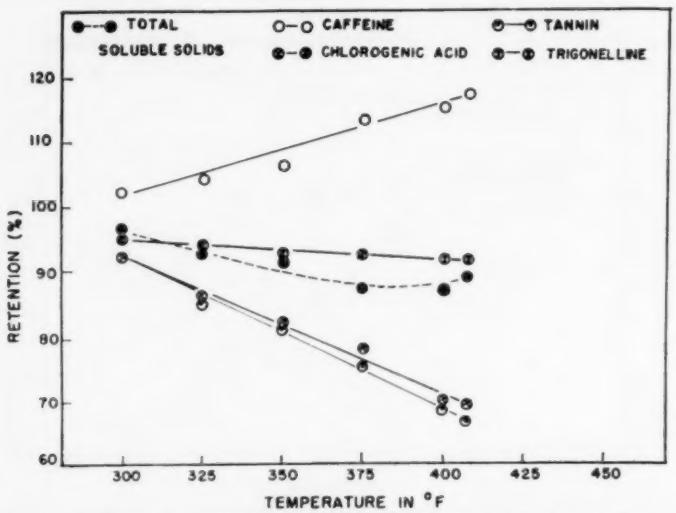

Figure 7. Effect of roasting temperature on Central American coffee.

Trigonelline (Figures 1-7) decreased inversely with temperature in the case of Santos, Maracaibo-Cuban, Medellin-Armenia, Peruvian, and Central American coffee samples. Temperature produced an immediate effect on trigonelline in Santos coffee, while the effect on this component in Central American coffee was negligible throughout the roasting cycle. Trigonelline

decreased immediately in the case of Post-Crop Colombian and Old-Crop Bucaramanga coffee, and then remained fairly constant until after 350° F was reached; it then decreased gradually to the end of the roasting cycle.

Chlorogenic acid and tannin (Figures 1-7) decreased progressively with temperature throughout the roasting cycle. The effect was most marked on these components in Post-Crop Colombian and Old-Crop Bucaramanga coffee samples; the least in Santos coffee.

The overall effect of temperature throughout the roasting cycle on the selected components of the seven types of coffee was different in every case. It is possible that this wide variation in temperature effects may be a contributing factor to quality differences *(15)* noted in roasted coffee.

SUMMARY

The effect of temperature at specific temperatures during the roasting cycle on selected components of whole bean coffee types, namely, total soluble solids, caffeine, trigonelline, chlorogenic acid, and tannin, has been investigated.

Seven different types of coffee, including Santos, Maracaibo-Cuban, Medellin-Armenia, Post-Crop Colombian, Old-Crop Bucaramanga, Peruvian, and Central American, were analyzed, and the per cent retention for each component noted.

Total soluble solids decreased gradually until 375° F. was reached, and then increased with temperature to the end of the roasting cycle.

Caffeine increased directly with temperature, the increase being gradual for Post-Crop Colombian, Old-Crop Bucaramanga, and Peruvian coffee. For Maracaibo-Cuban and Medellin-Armenia coffee, the change with temperature was immediate, followed by a gradual increase to the end of the roasting cycle.

Trigonelline decreased with temperature. For Post-Crop Colombian and Old-Crop Bucaramanga coffee, this component remained fairly constant, except for an initial drop, until after 350° F. was reached.

Chlorogenic acid and tannin decreased fairly rapidly with temperature throughout the roasting cycle.

Acknowledgment

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ELECTROPHORETIC ANALYSIS OF FLUIDS OBTAINED FROM MECHANICALLY DISRUPTED AND FROZEN FISH MUSCLE

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Process-induced changes in the colloidal structure of fish muscle constitute certain well-recognized quality problems. When fish muscle is frozen and subsequently thawed, for example, a cloudy, liquid phase called "drip" exudes from the muscle. The rate and degree of such deterioration in frozen fish are related not only to freezing and cold storage conditions but also to species differences.

As to the nature of drip, studies previously reported *(5)* noted a definite similarity between the protein composition of drip and low ionic strength extracts from fresh fish muscle. Thus, a comparison of low ionic strength extracts of fresh fish muscle and drip obtained by centrifuging thawed fish muscle revealed no differences as to the number of components present or their individual elctrophoretic mobilities. Although that fluid which exudes from fish muscle under the application of considerable external pressure (expressible fluid) reportedly contains small amounts of nuclear aggregates *(3)* and other unidentified components *(1)*, the proteins which predominate in such material are very similar to those extracted by a buffer solution of low ionic strength and neutral pH. Since the sarcoplasmic proteins are those which, for practical purposes, determine the protein composition of low ionic strength extracts, it may be assumed that the proteins of drip (or of expressible fluid) are essentially of intracellular origin.

Considerable histological evidence has been put forth attempting to relate the loss of such fluids resulting from the freezing of fish solely to physical damage to the extracellular structure of the muscle brought about by the formation of ice. Although such evidence generally demonstrates the effect of freezing on the physical state of tissue, little direct evidence is thereby obtained to relate the actual loss of fluids from thawed, disorganized tissue to simple cell rupture. Further, when it is considered that the protein concentration of drip is approximately only one-quarter of that of the intact sarcoplasm *(8)*, it would appear unlikely that the rupture of the sarcolemma by the mechanical force of freezing, in itself, fully explains the exudation of fluid from thawed tissue. It has been suggested *(5)*, rather, that denaturation of the fibrillar protein actomyosin by freezing, with the resulting contraction of muscle fibrils, is at least partially responsible for the liberation of the intracellular sarcoplasm. It would appear necessary that cell damage occur, however, in order to effect the release of the intracellular sarcoplasm, regardless of possible changes in forces stabilizing the weakly structured sol of the sarcoplasm or the fibrillar proteins.

In view of the uncertainty regarding the relative importance of cell rupture to the loss of fluids from fish muscle that has been frozen, it appeared desirable to further examine the question of cell damage. Thus, if cell rupture only affects but does not fundamentally cause the release of fluids from frozen

fish, it seemed possible that muscle fluids obtained by intentionally rupturing the tissue cells without freezing might prove different from fluids obtained from frozen muscle.

It was the object of the present work to compare the protein composition of fluids that could be obtained from mechanically disrupted fresh fish muscle with the protein composition of drip from frozen fish muscle and of whole sarcoplasm. The present paper is a summary of an electrophoretic study of such fluids.

EXPERIMENTAL

Materials. All of the work reported in this paper was carried out on fluids prepared from the white muscular tissue of quillback rockfish *(Sebastodes maliger)*. The whole fish were held for approximately 40 hours at 0° to 4° C., filleted and skinned, and the small amount of red, lateral tissue removed prior to sample preparation.

Two types of tissue fluids were prepared. One was obtained, as previously described *(6)*, by direct extraction of a tissue sample with an equal volume of phosphate buffer of ionic strength 0.05, pH 7.58, and centrifugation of the mixture. This fluid is referred to as a "low ionic strength extract". The other was obtained by centrifuging 50 g. of a tissue sample for 60 minutes at 4,000 × g at 0° C. The latter fluid is referred to either as "centrifuge liquor", if prepared from fresh fish muscle, or as "centrifuge drip", if prepared from frozen fish muscle. The frozen samples were prepared by placing both whole and mechanically disrupted fresh fillets (individually wrapped in aluminum foil; fillet weight, approximately ¼ lb.) directly on the plate of a contact-freezer (− 29° C., still air). These samples were held for from 1½ to 2 months at −29° C. and then thawed overnight in still air at 0° C.

Methods. The volume of tissue fluid recovered for a given sample was estimated by use of a graduated cylinder. The protein concentration of these fluids was determined by the biuret method *(7)*. Electrophoretic analyses of the tissue fluids were carried out as previously described *(6)*. The tissue fluids were dialyzed against phosphate buffer of ionic strength 0.05, pH 7.58, centrifuged at 24,500 × g, and diluted to below 4 mg. nitrogen/ml. with the final dialysate prior to electrophoretic analysis. Electrophoresis was conducted in a Perkin-Elmer Apparatus, Model 38A.

RESULTS

Typical electrophoretic diagrams of low ionic strength extracts obtained from intact, coarsely minced, and mechanically disintegrated fresh muscle are shown in Figure 1(a), 1(b), and 1(c), respectively. (Experimental details of the electrophoretic analyses and composition data calculated for the tissue fluids of Figure 1 are given in Table 1.) Intact

TABLE 1

Composition data [1] and details of electrophoretic analyses of tissue fluids diagrammed in Figure 1

Exp. No.	Electric field (v./cm.²)	pH	Duration of Electrophoresis (sec.)	Component, (% total area) 1	2	3	4	5	6	7
a	11.9	7.47	2190	1.8	4.0	12.0	23.1	23.1	16.4	19.6
b	11.9	7.47	2490	2.0	4.0	11.9	18.6	25.3	15.4	22.9
c	12.1	——	2400	5.3	6.2	17.7	18.7	14.6	16.8	20.6
d	11.9	7.47	2700	8.0		37.5	46.5		8.0	
e	11.8	7.45	2400	3.7	3.2	7.8	18.4	27.6	19.8	19.4
f	12.0	7.50	2700	2.9	3.5	9.4	22.4	25.3	13.5	22.9
g	12.0	——	2520	5.3	4.8	13.8	17.0	28.2	13.3	17.5
h	11.9	7.45	2220	3.5	3.2	10.9	24.3	26.8	14.8	16.5
i	11.9	7.51	2280	2.3	3.9	10.1	24.0	23.0	14.6	21.1
j	12.0	——	2700	2.8	7.1	10.8	20.9	18.5	19.7	20.3

[1] Calculated from ascending diagrams.

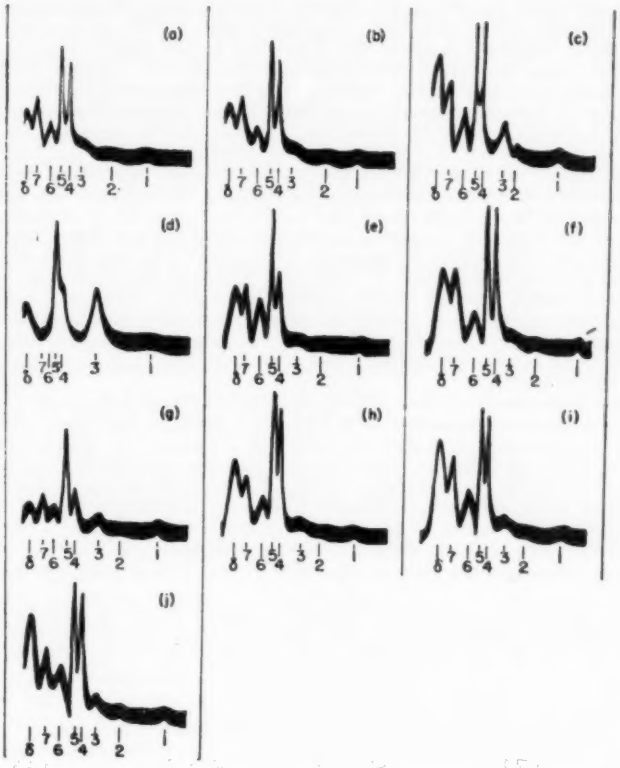

Figure 1. Ascending electrophoretic diagrams of tissue fluids.

(a) Ionic strength 0.05, pH 7.58 extract of intact fresh muscle.
(b) Ionic strength 0.05, pH 7.58 extract of coarsely minced fresh muscle.
(c) Ionic strength 0.05, pH 7.58 extract of mechanically disintegrated fresh muscle (Low speed blending).
(d) Ionic strength 0.05, pH 7.58 extract of mechanically disintegrated fresh muscle (High speed blending).
(e) Centrifuge liquor from intact fresh muscle.
(f) Centrifuge liquor from coarsely minced fresh muscle.
(g) Centrifuge liquor from mechanically disintegrated fresh muscle.
(h) Centrifuge drip from thawed intact muscle.
(i) Centrifuge drip from thawed coarsely minced muscle.
(j) Centrifuge drip from thawed mechanically disintegrated muscle.

muscle consisted of fillets cut only into small enough pieces (approximately 1 × 3 in.) to fit inside of a 100 ml. centrifuge tube. The minced muscle was prepared by passing the fillets once through a chilled, commercial food grinder equipped with a grinding plate having one-quarter inch diameter holes. Unless otherwise indicated, mechanically disintegrated muscle was prepared by blending minced muscle in a cold electric blender at low speed for approximately 5 minutes. With respect to their protein components, the low ionic strength extracts of intact and minced fresh muscle are very similar. A slight

difference was noted, however, in the diagram for a low ionic strength extract of disintegrated muscle. Thus, component 3 contributes more (18%) to the total area of the diagram for disintegrated muscle than it does for minced muscle (12%) and, in the former case, shows no tendency to migrate with component 4. In other respects, the diagrams appear to be quite similar. Figure 1(d) is also a diagram of a low ionic strength extract obtained from disintegrated fresh muscle. In this case, however, the muscle was disintegrated at high speed in the blender for 5 minutes prior to extraction. Here, component 3 is again distinct and widely separated from component 4 and contributes a very large portion (38%) to the total area of the diagram. In addition, components 6 and 7, which in previous extracts contributed appreciably (37%) to the total area of the diagrams, seem to be essentially absent (8%).

Typical electrophoretic diagrams of centrifuge liquor obtained from intact, minced, and disintegrated fresh muscle are shown in Figures 1(e), 1(f), and 1(g), respectively. It may be seen that the diagrams of centrifuge liquor obtained from intact and from minced fresh muscle are very similar and closely resemble the diagram for the low ionic strength extract of minced muscle. The diagram for centrifuge liquor obtained from the disintegrated muscle is different, however, in that component 3 again contributes more (14%) to the total area of the diagram than for the mince (9%) and shows no tendency to migrate with component 4.

Typical electrophoretic diagrams of centrifuge drip obtained from intact, minced, and disintegrated muscle are shown in Figures 1(h), 1(i), and 1(j), respectively. All of these diagrams are quite similar, although component 3 in the diagram for disintegrated muscle is perhaps slightly more distinct. In general, the migration characteristics of component 3 of these diagrams approximate those for extracts or centrifuge liquor of disintegrated tissue. Thus, component 3 of centrifuge drip diagrams is quite distinct and shows fair separation from component 4. It is interesting, however, that component 3 of centrifuge drip contributes less (11%) to the total area of the diagram than it does for any of the other tissue fluids, with the exception of centrifuge liquor from intact (8%) or minced (9%) tissue.

The percentage of the total sarcoplasmic protein recovered in each of the tissue fluids described in Figure 1 (based upon exhaustive extraction of minced muscle with an ionic strength 0.05, pH 7.58 phosphate buffer) is given in Table 2. Approximately 75%, 39%, and 54% of the total sarcoplasmic protein was obtained in low ionic strength extracts, centrifuge liquor, and centrifuge drip, respectively, from either minced or disintegrated tissue. From intact tissue, 9% and 40% of the total was obtained in the centrifuge liquor and centrifuge drip, respectively.

TABLE 2

Sarcoplasmic protein[1] in tissue fluids diagrammed in Figure 1

Type of tissue fluid	Treatment of muscle		
	Intact	Minced	Disintegrated
Low ionic strength extract	[2]	74	76
Centrifuge liquor	9	41	37
Centrifuge drip	40	54	54

[1] Expressed as percent of total sarcoplasmic protein of the muscle recovered in the tissue fluids. Total sarcoplasmic protein N = 4.87 mg./g. tissue, based upon exhaustive extraction of minced muscle with ionic strength 0.05, pH 7.58 phosphate buffer.
[2] Not determined.

DISCUSSION

The electrophoretic diagrams for tissue fluids prepared by various methods show the striking similarity of their protein composition, despite differences in the relative amounts of the sarcoplasmic fraction recovered. Thus, although approximately only half of that amount obtained by low ionic strength extraction of fresh tissue was recovered by direct centrifugation,

the number and relative amount of the components present appear to be essentially the same. In addition, when the fresh tissue was frozen, thawed, and centrifuged, although a somewhat larger amount of the sarcoplasmic fraction was obtained in the centrifuge drip (compared with the centrifuge liquor from fresh tissue), little overall difference was noted in the composition of the tissue fluids. It would therefore appear suggestive that very weak forces stabilize the intracellular sarcoplasm, such that when cell rupture is induced, a relatively large proportion of the fluid cellular contents are rather readily shed into the muscle exudate. This is in agreement with the findings of Love *(4)*, who concludes that the muscle cell plasma found in expressible fluid of iced cod is derived from the ends of cells cut at the surface of the pressed fillets. The protein concentration of centrifuge liquor is of the order of 5%, however, which is only approximately one-fourth of the protein concentration of the intact sarcoplasm *(8)*. An appreciable dilution of the sarcoplasm therefore occurs even under conditions where denaturation of the fibrillar protein actomyosin is avoided. Thus, it would appear that centrifuge liquor or expressible fluid of iced cod do not reasonably represent intact sarcoplasm.

It has been suggested *(5)* that denaturation of actomyosin by freezing through a dehydration process is partially responsible for the liberation and dilution of sarcoplasm. In the case of centrifuge liquor from fresh fish, however, it is difficult to imagine a parallel situation; and as Connell *(1)* points out, a significant dilution of the sarcoplasm by admixtue with intercellular fluid seems to be very unlikely. Connell *(1)* suggests, rather, that the water released in the syneresis of the muscle fibrils during rigor probably accounts for this dilution effect. In this sense, a parallel may be drawn with the denaturation of the fibrillar proteins by freezing.

Although only minor over-all differences were noted in the protein composition of the tissue fluids of the present study, certain differences in one of the components are of interest. Thus, component 3 of Figure 1 appears to have features characteristic of the manner in which the muscle tissue was treated. Component 3 comprises about 11% of the proteins of centrifuge drip from intact or minced tissue and about 9% of the proteins of centrifuge liquor from intact or minced fresh tissue. After the tissue has been frozen, although component 3 thus shows little change in relative amount, it consistently migrates as a more distinct entity, rather than being attracted to component 4. This effect is most striking in any one type of tissue fluid which may be obtained from fresh muscle tissue of varying degrees of disintegration. Component 3 of low ionic strength extracts, for example, becomes progressively more distinct and it contributes more (from 12 to 18%) to the total area of the diagram as cell destruction becomes more complete. Severe disintegration of the tissue yielded about 38% of component 3, although in this case it is considered that other components were denatured by the extremely severe treatment. Component 3 of centrifuge liquor also shows a progressive increase in relative amount with cell damage (from 8 to 14%).

It was first felt that these observed differences in the relative amounts of component 3 could be due to manipulative artifacts or aggregates *(2)*. A consideration of the differences in migration characteristics and resulting

shapes of the peaks when the tissue is either disintegrated or frozen, however, suggests that although component 3 of the sarcoplasm very likely undergoes a change when tissue is frozen or mishandled, it apparently remains as a distinct entity. In addition, since all tissue fluids were centrifuged at 24,500 x g prior to analysis, it appears unlikely that component 3 could be regarded as an aggregate.

It is of interest that Connell *(1)* reports a component in electrophoretic diagrams of the expressible fluid of fresh cod muscle which appears to possess mobility and composition characteristics similar to those of component 3 reported here. Thus, Connell *(1)* notes about a 7% increase in area in the region of this component when comparing extracts of minced flesh made at low ionic strength with expressible fluids obtained from thawed fish. Connell *(1)* suggests that this increase seems to be due to the result of heavy pressure on the muscle, because such an increase is not observed in the fluid obtained by centrifuging minced fresh muscle.

In general then, it would appear that cell rupture, in itself, can effect the release of considerable intracellular sarcoplasm, the nature of which is dependent somewhat upon the manner in which the tissue is treated. Thus, it has been shown that the escape of one component into the muscle exudate becomes more complete as cell rupture increases, although the release of this component is likely dependent upon changes in forces stabilizing the weakly structured intracellular sol. The exudation of drip from thawed fish is therefore very likely due not only to cell rupture, caused by the mechanical force of freezing, but also to a hysteresis of the fibrillar proteins and a change of forces stabilizing the sarcoplasm.

SUMMARY

The protein composition of fluids that could be obtained from mechanically disrupted fresh fish muscle have been compared electrophoretically with drip from frozen fish muscle and with the whole sarcoplasm of fish muscle.

It was determined that rupture of the cells of fresh tissue by mechanical means liberates a tissue fluid which contains the same number of protein components as are contained in drip obtained from frozen tissue and in low ionic strength extracts (sarcoplasm) from fresh tissue. Differences in mobility characteristics for one protein component and the relative contribution of this component to the total protein composition, however, were observed. Such differences were attributed to the degree to which the muscle cells were ruptured and to changes in forces stabilizing the weakly structured sol of the sarcoplasm by freezing or manipulative means.

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FLAVOR COMPOUNDS FROM COMMERCIAL EVAPORATED MILK[a]

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Evaporated milk possesses a characteristic flavor resulting from its sterilizing heat treatment. Various approaches have been used in attempts to identify the compounds responsible for this flavor. In most instances the materials have been subjected to very rigorous heat treatments, on the assumption that these would yield greater concentrations of the same compounds developed by commercial sterilization. A whole series of compounds have been reported to occur in heated milk, including acetaldehyde, furfural, hydroxymethyl furfural, acetal, maltol, methyl glyoxal, acetic, lactic, formic, propionic and pyruvic acids, furfuryl alcohol, etc. *(8)*; acetaldehyde has also been reported to occur in unheated skim milk *(5)*. Yet, our knowledge of the specific functional entities responsible for the flavor of commercial evaporated milk is still meager.

The present study was conducted to isolate and identify volatile components from commercial evaporated milk, using a continuous steam-distilling assembly to minimize additional heat damage to the product.

MATERIALS AND METHODS

Steam distillation of the milk. Two hundred cans of evaporated milk processed about 3 months earlier and purchased at a local market were steam distilled in the assembly shown (Figure 1). Continuous steam distillation was performed for several 12-hour periods, and the assembly was dismantled and washed after each run. The average volume of milk distilled in a run was about 3.5 liters.

The apparatus consisted of a stripping chamber that permitted rapid removal of volatile materials with practically no additional heat damage to the product. Steam generated in a 3-neck flask was introduced into the bottom of the chamber, countercurrent to the milk, which was continuously siphoned from an elevated reservoir. The vapors were conducted through an entrainment separator to a refrigerated water condenser and collected. Effluent milk was discharged through the vapor-blocking trap on the stripping chamber and collected for reflectance measurements.

Attached to the outlet of the condenser in the early runs was a gastight absorption train, consisting of a dry-ice trap, a drying tube filled with granular lead acetate, and 2 gas-scrubbing towers containing, respectively, 10 ml. of 3% aqueous mercuric chloride and 10 ml. of 1% isatin in sulfuric acid. Dateo *et al. (2)*, working with cooked cabbage, detected hydrogen sulfide using solid lead acetate, and mercaptans using 3% mercuric chloride solution. Isatin in sulfuric acid reacts with mercaptans to give a green color *(1)*. Nitrogen injections were made with a hypodermic syringe through the serum cap at 10-minute intervals to sweep into these towers any minute quantities of non-condensable vapors that might be present.

Standard plate counts were taken on unstripped milk at the beginning and end of three of the runs, with no significant bacterial growth observed. Heat damage was assessed by comparing the reflectance of the effluent milk with that of commercial evaporated milk diluted to the same extent. Reflectance was measured by a previously described procedure *(4)*. The data in Table 1 indicate clearly that there was no further browning during the operation.

[a] Report of a project supported in part by funds from the California Dairy Industry Advisory Board.

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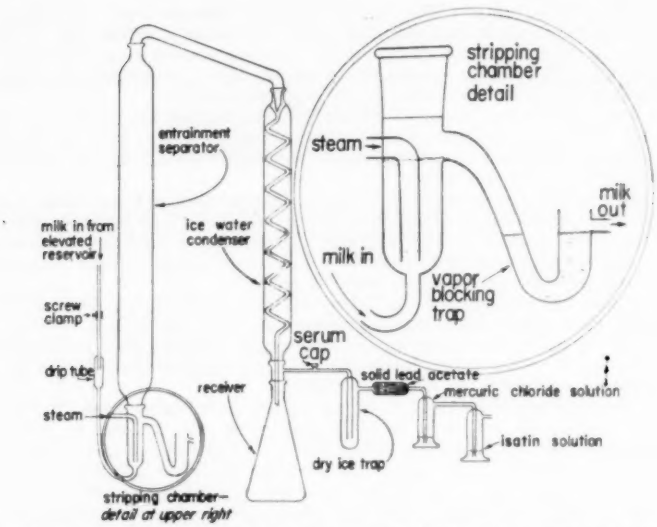

Figure 1. Assembly used in the steam distillation of evaporated milk.

Concentration of the distillate. The steam distillate (about 18 liters) had the flavor typical of evaporated milk, but failed to respond to qualitative tests for functional groups. It was concentrated about 40:1 in a modified Oldershaw bubble-plate column. The concentrate possessed a much more intense odor than the original steam distillate, but was still too dilute to react for functional groups; it did give a strong positive test for ammonia *(3)*.

Extraction procedure. The concentrate was saturated with C.P. sodium chloride, placed in a separatory funnel, chilled to about 4° C., and extracted with U.S.P. ethyl chloride. Each 200-ml. portion of the concentrate was extracted with two 35-ml. portions of ethyl chloride. The solvent phase was collected and dried over anhydrous magnesium sulfate for 3 days.

Infrared spectroscopy. After evaporative removal of the solvent in an ice-water bath a very small drop of a volatile yellow oil was left which had a stale, cereal-like odor. This material was kept in dry ice for a few hours before infrared study in a Perkin-Elmer Model 21 infrared spectrophotometer. Because of failure to vaporize enough of the sample to obtain a gas phase spectrum, the material was taken up in carbon disulfide and placed on a sodium chloride prism as a thin film, and an informative spectrum was obtained.

Preparation and isolation of derivatives. Freshly prepared 2,4-dinitrophenylhydrazine reagent *(9)* was added to the extract after removal of the solvent, and the mixture was allowed to stand overnight. The material was extracted with petroleum ether and dried

TABLE 1

Reflectance measurements of effluent milk

Batch No.	Milk used	Milk discharged	Water incorporated	Reflectance of dischargd milk	Reflectance of evaporated milk diluted accordingly
	(ml.)	*(ml.)*	*(ml.)*	*(%)*	*(%)*
1	3,420	5,490	2,070	51.5	51.0
2	3,730	5,610	1,880	50.0	51.0
3	3,660	5,670	2,010	50.7	51.5

over anhydrous sodium sulfate. The 2,4-dinitrophenylhydrazones were resolved on a 9 × 150-mm. bentonite-celite column according to the procedure of White *(10)*. Columns were developed under 5-6 p.s.i. nitrogen pressure. The best separation was obtained when ethyl ether and acetone (25:1) were used as the solvent. The various zones were dug out of the column and the derivatives eluted with absolute methanol.

To detect fractions that might be present in concentrations too minute for visual detection, the column was mounted on an automatic fraction collector, and 1-ml. fractions collected. The fractions obtained were scanned in the regions 210-225 mμ and 340-390 mμ on a Beckman DU spectrophotometer.

Fractions were re-chromatographed to ensure homogeneity, and recrystallized in a minimum of dilute ethyl alcohol. Melting points were determined both by the conventional capillary tube and the microscope stage methods. The accuracy of the latter was improved by limiting the heating increment to about 0.1° C./min. and fitting a bored cork on the objective of the microscope to limit heat losses by radiation.

Paper chromatograms were prepared by inoculating 7λ quantities on Whatman No. 1 paper, stapling into a cylindrical form, and placing in screw-cap Mason jars. The solvents used were (a) 75:25 hexane methanol, and (b) hexane saturated with β-phenoxyethyl alcohol. For use in the latter solvent system, the paper was immersed in 10% phenoxyethyl alcohol in acetone and dried in air before inoculating.

Trial with radioactive lactose. A 400-mg. portion of uniformly labeled lactose was dissolved in 4 ml. of distilled water at 45° C. and added, with stirring, to 760 ml. of unsterilized evaporated milk. Two 14½ oz. cans were filled with the material, soldered, and sterilized 15 minutes at 242° F. by a procedure previously described *(4)*. Because the milk obtained for these trials was not as heat-stable as expected, it became quite viscous after sterilization, and some coagulation occurred in the steam stripper. To prevent this, the proteins were removed by adding an equal volume of 15% trichloroacetic acid, and the filtrate neutralized with 33% NaOH. The whey was steam-distilled and the distillate extracted with ethyl chloride. The 2,4-dinitrophenylhydrazones were prepared and chromatographed as described above, and the colored zones of the column were recovered and eluted with absolute methanol. The eluates were evaporated to dryness at room temperature with a stream of dry air, and radioactivities were determined in a Tracerlab SC-16 windowless flow counter with a Tracerlab 1000 scaler. Subsequently, they were taken up in 95% ethanol for spectral analysis.

Trial with radioactive alanine. A few milligrams of 2-C^{14} alanine were dissolved in 0.5 ml. of 0.1 *N* HCl and purified by paper chromatography, using 80% phenol in water as the solvent. Simultaneously, non-radioactive alanine was chromatographed in another region of the same paper and detected with ninhydrin reagent. A knowledge of its position, coupled with Geiger-counter scanning of the chromatogram, permitted recovery of the purified radioactive alanine. The paper strip was cut into small pieces and immersed in 8 ml. of 0.05 *N* NaOH for elution. This material was directly added to 1520 ml. of unsterilized evaporated milk,[b] stirred well, and placed in four 14½ oz. cans. The rest of the procedure was identical to that used in the lactose trial.

RESULTS AND DISCUSSION

After several weeks of steam-distillation there was no turbidity in the mercuric chloride solution, nor had any green color developed in the isatin. This indicated the absence of mercaptans. The lead acetate trap, however, after a few hours of distillation, showed considerable blackening, indicating that hydrogen sulfide, long recognized to occur in heated milk, is also present in evaporated milk.

Because the amount of extracted material obtained from the steam distillate of the milk was so small, sacrificing even a portion for exploratory functional group tests was not desirable. An infrared spectrum of the extract was obtained to show which functional group(s) required further work. The absorption bands of the spectrum were interpreted as follows:

[b] There was no increase in the pH of the milk after addition of the tracer.

2925, 2853 — aliphatic C-H stretching vibration, probably CH_2;

1720 — 1750 carbonyl stretching vibration, width of band suggests more than one carbonyl type;

1468, 1377 — CH_3, CH_2 bending vibrations, further evidence of aliphatics

1008, 738 — probably the most useful of the remaining bands for checking any proposed chemical identification;

1260, 1221, 1148, 1072, 910, 805 — these bands are distinct and real; they cannot be assigned to CS_2 or ethyl chloride.

The absence of absorption at 3000-3050 cm^{-1} indicates that no appreciable olefin or aromatic C-H is present. The band at 1221 cm^{-1} probably indicates aliphatic ketones, since aromatic aldehydes have been ruled out.

Because the extract had the flavor typical of evaporated milk, and carbonyl was the only functional group present, the characteristic flavor of evaporated milk is probably due to a mixture of heat-generated carbonyl compounds. Although the literature reports many compounds isolated from heated milk *(8)*, no evidence is available as to which type(s) are responsible for the flavor of evaporated milk.

The extract was allowed to react with 2,4-dinitrophenylhydrazine, and two hydrazone fractions were obtained from chromatographic resolution on celite-bentonite columns: a wide yellow band ("A") and a narrow orange band ("B"), with B developing about twice as fast as A. After checking for chromatographic homogeneity an attempt was made to recrystallize each fraction from ethanol-water. Fraction A gave yellow needles melting at 151-154° C., but attempts to recrystallize fraction B were unsuccessful.

Paper chromatography in 2 different solvents indicated that fraction A was the same as the 2,4-dinitrophenylhydrazone of acetaldehyde (Figs. 2 and 3). This was confirmed by mixed melting points, co-chromatography on bentonitecelite, and spectral data (Fig. 4). Fraction B showed a peculiar ultraviolet spectrum, with two peaks in the 320-360 mμ region (Fig. 5). When the carbonyl moiety of fraction B was regenerated by heating in levulinic acid *(7)*, the odor liberated was described by various people as ranging from yeasty to "reminiscent of a bakery".

In the later phases of this work, a new procedure became available for chromatographic separation of 2,4-dinitrophenylhydrazones. This method uses columns of nitromethane-saturated Supercel,[c] and nitromethane-saturated hexane as the developing solvent, and has permitted establishment of relative rates for a series of known compounds *(6)*. When this technique was applied to the 2,4-dinitrophenylhydrazone mixture, three fractions were evident. These were the major, slow-moving acetaldehyde fraction, and 2 minor, faster

[c] Johns-Manville, New York, N. Y.

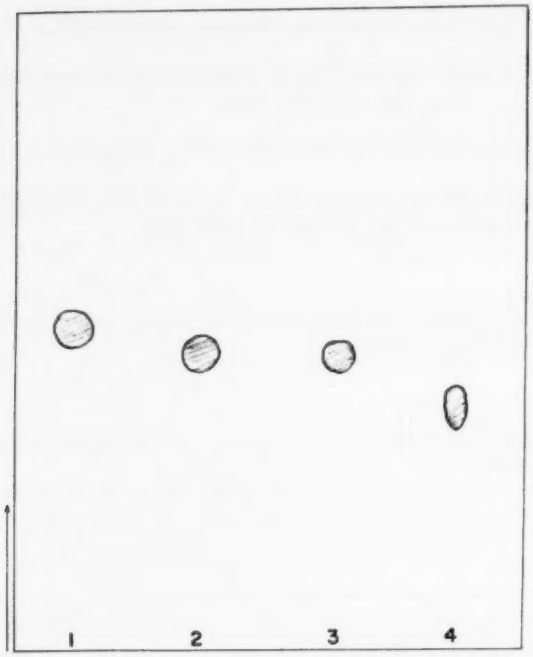

Figure 2. Ascending paper chromatogram of some 2,4-dinitrophenylhydrazones in hexane-methanol (75:25 by volume). (1) Propanal; (2) Fraction A; (3) Acetaldehyde; (4) Furfural.

bands, with rates coincident with those reported for 2-heptanone and 2-pentanone *(6)*. This tentative identification was confirmed by paper chromatography of these fractions and the hydrazones of synthetic 2-pentanone and 2-heptanone, using hexane as the solvent. Addition of 0.5 p.p.m. of each of these synthetic ketones to unsterilized milk produced a flavor suggestive of evaporated milk flavor.

The origins of the acetaldehyde and of fraction B were studied with radioactive additives. The most probable precursors are lactose and alanine,

TABLE 2

Radioactive assay of the 2,4-DNPH recovered

Description of fraction	Specific activity of blank planchet		Specific activity of planchet with material	
	Counts/min.	Time of count	Counts/min.	Time of count
		(min.)		*(min.)*
Fraction A (alanine run)	20.5	10	58	30
Fraction A (lactose run)	22	10	41	30
Fraction B (alanine run)	23	10	23	20
Fraction B (lactose run)	17	10	24	35

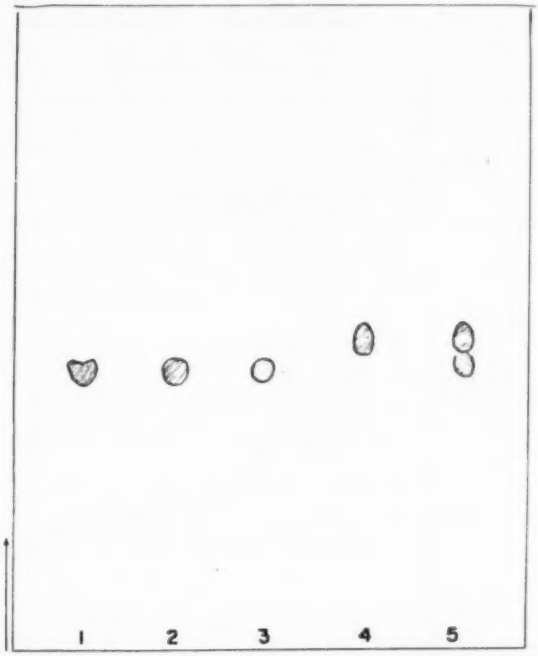

Figure 3. Ascending paper chromatogram of some 2,4-dinitrophenylhydrazones in hexane saturated with −phenoxyethanol. (1) Acetaldehyde + Fraction A; (2) Acetaldehyde; (3) Fraction A; (4) Propanal; (5) Propanal + Fraction A.

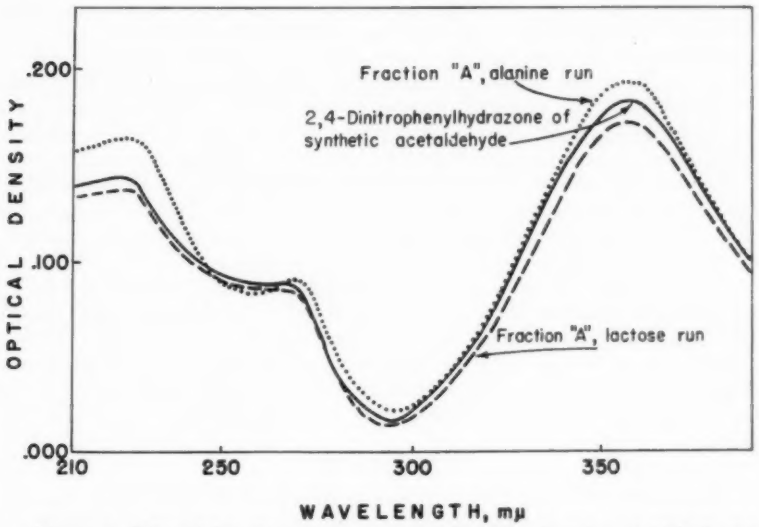

Figure 4. Ultra-violet absorption spectra of Fraction A and acetaldehyde 2,4-dinitrophenylhydrazone.

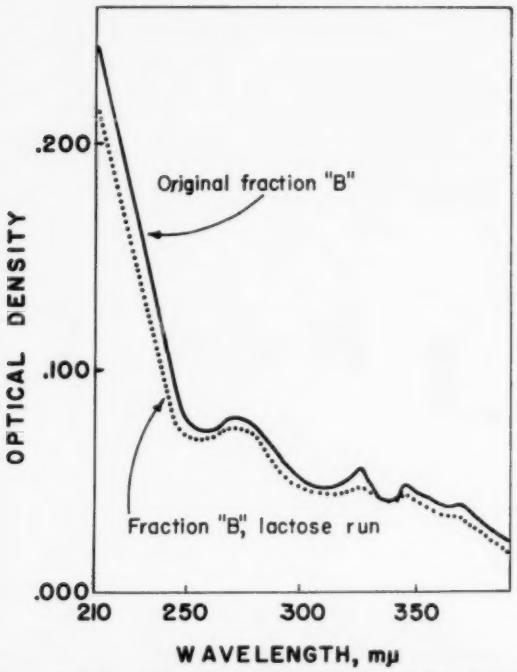

Figure 5. Ultra-violet absorption spectrum of Fraction B.

and parallel trials were conducted with evaporated milk to which was added uniformly labeled lactose in one case, and 2-C ^{14} alanine in the other. Results are shown in Table 2.

In Figure 4, the ultraviolet spectra of the 2,4-dinitrophenylhydrazones of acetaldehyde and of the A fractions from the two radioactive runs are shown to be identical. Similarly, fraction B from the labeled-lactose run exhibits the same spectrum as the original fraction B (Fig. 5).

Data in Table 2 show that both lactose breakdown and oxidative deamination and decarboxylation of alanine contribute to acetaldehyde formation in evaporated milk. Acetaldehyde might also arise from alanine produced by the decarboxylation of aspartic acid, and by oxidation of lipid materials.

When aqueous solutions of amino acids were heated 15 minutes at 242° F., the carbonyls produced indicated that the Strecker degradation had occurred, since the reaction was accelerated by additions of ascorbic acid. This is probably the mechanism for the production of acetaldehyde from alanine in evaporated milk. It seems logical that other amino acids would also undergo such degradation and yield carbonyls of considerable organoleptic significance.

SUMMARY

Evaporated milk was steam-distilled in an assembly which minimizes additional heat damage, and the distillate, which possessed the typical flavor and

odor of evaporated milk, was concentrated and extracted with ethyl chloride.

Infrared studies indicated that the material extracted was a mixture of carbonyls. The 2,4-dinitrophenylhydrazone mixture was resolved into three fractions by column chromatography using two different procedures. They were identified as acetaldehyde, 2-pentanone and 2-heptanone, and the identification confirmed by paper chromatography.

Results of tracer studies showed that acetaldehyde is derived both from lactose breakdown and deamination and decarboxylation of alanine. Lactose was also found to be the precursor of another carbonyl fraction which could not be completely characterized.

Hydrogen sulfide was evolved during the steam distillation, but there was no evidence of mercaptans.

Acknowledgments

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SOME TECHNIQUES FOR EVALUATING THE FLAVOR OF PROCESSED MEAT

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The flavors developed during cooking of various meats have been studied by several investigators. A review of exploratory studies designed to indicate the nature of the flavor of raw and boiled beef, lamb, pork, and chicken was prepared by Crocker *(9)*. He observed that the flavor of fresh raw meats appeared to be mostly in the juices which could be extracted. All of the meats investigated have a weak, sweet, salty, and blood-like taste. Superimposed on this taste are flavors distinctive of the species, food, and environment of the animal. Raw meat fibers, with tissue juices removed, had no detectable odor, and on chewing, no taste. When the raw fibers of fresh beef were heated (boiled) for a few minutes, a marked flavor characteristic of roast beef developed which was released during chewing. The flavor was identified primarily as an odor since only a trace of taste was noted when the nostrils were closed. Sensory tests indicated that most, if not all, of the cooked meat flavor came from meat fiber and that little, if any, contribution came from bones, marrow, or fat.

A distillate obtained by boiling defatted beef contained ammonia and hydrogen sulfide which could be identified by odor. *Bouthilet (3, 4, 5)* in a series of three articles on chicken flavor, demonstrated the presence of a sulfur-containing organic compound of importance to chicken flavor. Pippen, Campbell and Eyring *(16)* and Pippen and Eyring *(17)* found that the volatile nitrogen present in chicken broth distillate consisted entirely of ammonia. Sulfur present in freshly prepared broth distillate was found to consist entirely of hydrogen sulfide as shown by the agreement between hydrogen sulfide and total sulfur. No mercaptan sulfur was found, and within the limits of experimental error all sulfur was accounted for as hydrogen sulfide.

The objectives of this investigation were to develop chemical techniques for evaluating the flavor of processed meat and to correlate these techniques with sensory evaluation tests.

EXPERIMENTAL AND RESULTS

Materials. Enough meat to yield 100 and 200 pounds of lean trimmings was obtained from at least 2 beef, 2 pork, and 3 lamb carcasses of good quality and of known history. This meat was purchased from an inspected packing plant. Each meat product was kept under adequate refrigeration and was divided into 2 lots. Sodium chloride was added to one lot to give a salt content of 1.5%. Some canned meat products and canned products containing meat prepared by commercial companies were obtained from Dr. E. W. Turner of the Quartermaster Food and Container Institute. These products were stored until used at room temperature. They were frozen in the cans prior to the test, opened, and ground 3 times while in the frozen state.

Methods. The salted portion of each meat (lean trimmings) was divided into 3 lots; 2 of these were processed to give duplicate runs of processing as indicated in Table 1.

TABLE 1

Some chemical characteristics[1] of meat products, steam distillates obtained from them, and food acceptance evaluations

Type of Product	Dry matter	Ether extract	Protein N × 6.25	Steam distillates Mg./100 g. of prod		pH in first receiver	Mean food acceptance score
				Hydrogen sulfide[2]	Ammonia		
	%	%	%				
Fresh beef luncheon meat not processed	43.2	23.9	16.8	0.3	4.0	9.3	——
Salted beef luncheon meat not processed	43.8	25.0	15.1	0.2	5.5	9.4	——
Salted fresh beef cooked as meat cakes in pressure saucepan for 5 min. at 120° C. (248° F.)	45.3	19.1	21.3	0.5	4.3	9.6	7.4
Beef luncheon meat heat processed 117 min. at 113° C (235° F) ($F_0 = 3.7$)	40.6	23.2	15.2	0.8	5.3	9.3	5.8
Salted beef luncheon meat, heat processed 117 min. at 113° C. (235° F.) ($F_0 = 3.2$)	43.2	23.2	14.8	0.8	6.2	9.5	5.7
Salted beef luncheon meat heat processed 138 min. at 113° C (235° F) ($F_0 = 6.0$)	43.3	23.7	14.9	0.8	6.8	9.4	5.4
Beef luncheon meat heat processed 138 min. at 113° C. (235° F) ($F_0 = 7.3$)	43.3	23.9	17.9	0.9	7.5	9.8	5.7
Raw lamb salted	39.4	20.7	15.1	0.4	4.1	6.5	——
Salted fresh lamb cooked as meat cakes in pressure saucepan for 5 min. at 120° C (248° F)	40.2	10.7	25.3	0.5	4.3	9.5	7.5
Salted lamb heat processed 117 min. at 113° C (235° F)	37.9	20.3	13.7	0.7	9.2	9.2	5.3
Lamb no salt heat processed 138 min. at 113° C (235° F)	40.4	23.1	14.3	0.9	10.8	9.2	5.2
Salted lamb heat processed 138 min. at 113° C (235° F)	38.9	20.1	14.1	1.0	9.2	9.2	5.2
Raw pork salted	43.8	24.7	15.6	0.2	2.3	7.8	——
Salted pork pressure cooked for 5 min. at 120° C (248° F)	47.6	22.1	21.8	0.6	3.7	9.2	8.6

TABLE I (continued)

Type of product	Dry matter	Ether extract	Protein N × 6.25	Steam distillates Mg./100 g. of prod		pH in first receiver	Mean food acceptance score
				Hydrogen sulfide [2]	Ammonia		
	%	%	%				
Salted pork heat processed 138 min. at 113° C (235° F)	42.2	24.3	12.9	1.0	6.5	9.3	6.1
Salted pork heat processed 138 min. at 113° C (235° F)	44.0	25.4	14.0	1.0	8.2	9.2	5.2
Pork not salted heat processed 138 min. at 113° C (235° F)	43.1	26.1	14.1	1.2	8.5	9.7	6.4
Luncheon meat (x)	37.8	15.4	16.0	1.2	6.9	9.8	3.1
Beef steaks (x)	37.9	7.2	24.9	1.1	7.9	9.7	4.1
Pork steaks (x)	42.5	24.8	13.9	0.4	8.6	10.2	4.1
Ham chunks (x)	43.4	18.5	17.5	0.8	8.5	9.8	4.3
Boned chicken (x)	34.3	11.2	19.8	0.7	8.8	9.9	5.1
Boned turkey (x)	36.7	13.4	20.3	0.4	10.5	6.8	4.8
Beef hamburger (x)	42.4	14.5	23.9	0.9	8.8	9.7	2.0
Tuna and noodles with vegetables (x)	30.6	10.3	10.1	0.1	2.4	7.1	6.2
Meat balls and spaghetti with tomato sauce (x)	27.3	5.3	9.9	0.1	2.4	5.5	4.5
Beef stew (x)	20.0	5.5	7.9	0.4	3.8	8.6	6.0
Corned beef hash (x)	32.3	5.2	10.4	0.3	5.2	9.2	4.1
Chopped ham and eggs (x)	34.5	15.7	13.7	0.5	10.1	9.6	3.1

[1] Average values reported after designated treatment.
[2] Volatile sulfure compounds as hydrogen sulfide.
(x) Products obtained from Quartermaster Food Container Institute.

Equipment for the measurement of heat penetration as described by Ecklund *(10)* was used in this study. Sterilizing values (F_0) were estimated by nomogram of the method of Olson, Stevens, and Roberts as described in a publication of the American Can Company *(1)*. Lamb and pork products were treated essentially like beef products except that they were processed in larger cans. All processed and unprocessed meat was frozen and kept in the frozen state until chemical or food acceptance tests were conducted.

Dry matter. Dry matter determinations were made by drying ground meat samples in a vacuum oven for 15 hours at 65° C. or until constant values were obtained.

Ether extract determinations were conducted by placing dry samples from the vacuum oven in thimbles in a Goldfish extraction apparatus and extracting with diethyl ether for 5 hours or until constant values were obtained.

Total nitrogen. Total nitrogen was determined by a slight modification of the Kjeldahl method described in *Official Methods of Analysis* of the Association of Official Agricultural Chemists (A.O.A.C.) *(2)*.

Peroxides and free fatty acids. Peroxides and free fatty acids determinations were made on fresh samples of beef, lamb, and pork by the method of Rockwood, Ramsbottom and Mahlenbackey *(19)*. The fat in the fresh meat samples contained about 0.2% free fatty acids, calculated as oleic acid. Peroxides were not detected in the fresh samples. After the meat was processed, the concentration of free fatty acid reached a level of nearly 1%.

Amino acids. Extraction of defatted samples of heat-processed beef luncheon meat with methanol for one hour, and evaporation of the methanol gave an extract which when taken up in distilled water possessed a cooked beef flavor. The dried residue was

quite tasteless. A positive test for amino nitrogen in the water solution was obtained by the Cocking and Yemm *(8)* modification of the method of Moore and Stein *(14)*. Amino acids in the extracts were demonstrated by unidimensional descending paper chromatography using butanol, acetic acid, water (4:1:5) as developing agents. Where necessary, the two-dimensional chromatographic procedure of Levy and Chung *(13)* was employed for identification of the amino acids. Of the 10 different spots on the chromatograms, 9 were tentatively identified as the amino acids: glutamic, aspartic, arginine, histidine, valine, tyrosine, glycine, leucine and/or isoleucine. Other amino acids may have been present in smaller amounts.

Volatile compounds. Since the flavor of meats appears to be associated with volatile compounds, a rapid steam distillation procedure was developed. A 50-g. sample of ground and thoroughly mixed meat was placed with 70 ml. of water in a 250 ml. Claissen flask fitted with a thermometer and steam inlet tube. The arm of the Claissen flask was connected with glass tubing to an all glass condenser clamped in a vertical position. The outlet end of the condenser was connected to a perforated polyethylene tube which dipped into 50 ml. of distilled water placed in a narrow 250 ml. wide-mouth Erlenmeyer flask, which served as the first receiver. The flask was connected to the condenser by a two-hole rubber stopper. A glass tube was passed through the rubber stopper and was connected to a filter stick which dipped into 50 ml. of distilled water placed in a 100 ml. test tube. Both receivers were cooled in an ice-water bath. The meat-water mixture was heated over a Bunsen burner to boiling in about 10 minutes, after which the burner was turned off and steam from a low pressure reservoir was introduced through the inlet tube. Steam was introduced slowly at first, but at an increasing rate during the distillation until 200 ml. of distillate were collected. The total heating time was 30 minutes. The pH of the distillate and water in the first trap was determined, and the material from the first and second traps was combined making a volume of 300 ml. Determinations for volatile sulfur and nitrogen compounds were promptly made on aliquots of the distillate. If the distillate was allowed to stand, lower values were obtained for these compounds.

Hydrogen sulfide. Hydrogen sulfide was determined colorimetrically by a modification of the methylene blue method described by Jacobs *(12)*. Values of color readings of standard sulfide solutions were used as a basis in computing the sulfide concentration in the distillates. Reliable results with the methylene blue procedure can be obtained only if the concentration of hydrogen sulfide is below 5 meq. per ml. Distillates from meat products seldom exceed 2 meq. per ml. of hydrogen sulfide.

Organic sulfhydryl groups. Tests for organic sulfhydryl groups by the procedure of Boyer *(6)* were made on steam distillates of several samples, but no positive tests were obtained under the conditions of these experiments.

Ammonia. Ammonia in the distillate was determined using the Nessler's reagent of Bock and Benedict according to Hawk, Oser and Summerson *(11)*. Ninhydrin reagent produced a blue color with the distillates. Ammonia also reacts with ninhydrin reagent to give a color similar to that produced by amine nitrogen compounds, but the color developed is 30 to 60% as much as that which develops with the same weight of nitrogen in the form of glutamic acid or other alpha amino acid. It appeared that the blue color produced by ninhydrin reagent was primarily a result of the reaction with ammonia, as attempts to detect amines other than amino acids by other methods gave negative results.

With few exceptions, all determinaitons were made in duplicate and in quadruplicate on many samples. All procedures were checked with appropriate blanks and standards.

Other components. Trace amounts of organic acids were detected in steam distillates, but attempts at identification were without positive results.

Small amounts of the 2, 4-dinitrophenyl hydrazones of carbonyl compounds were isolated from steam distillates, but not identified. Pippen *et al. (18)* identified many carbonyl compounds from cooked chicken.

Statistical analyses. Statistical analyses of values obtained for ammonia and hydrogen sulfide in distillates were made by the procedures of Cochran and Cox *(7)* (Table 2).

Food Acceptance Tests. For purposes of comparison with canned meats some samples of the raw meat were cooked in a pressure saucepan (Presto) for 5 minutes at 120° C. A few samples were broiled in a Westinghouse oven for 10 minutes. All products were tasted at room temperature for flavor, texture and juiciness by a taste panel of 8 to 13

TABLE 2

Analysis of variance for hydrogen sulfide and ammonia content in steam distillates of salted and non-salted lamb, pork, and beef luncheon meat processed for various periods of time

Source of variation	Degrees of freedom [1]	Mean squares					
		Lamb		Pork		Beef	
		Hydrogen sulfide	Ammonia	Hydrogen sulfide	Ammonia	Hydrogen sulfide	Ammonia
Replications	3	0.0120	0.0970	0.0031	0.0026	0.0002	0.0001
Time	3	0.7263 [2]	82.7425 [2]	1.0452 [3]	66.1956 [2]	0.3338 [2]	5.5084 [4]
Salt	1	0.0830	0.5278	0.0004	4.2122	0.0361	0.2165
Time x salt (error a)	1	0.0558	4.0994 [2]	0.0547	1.7488 [2]	0.0071	1.1973 [2]
Error "b" [5]	21	0.0196	0.1022	0.0035	0.0474	0.0008	0.0023
Total	31						

[1] Degrees of freedom for beef 1, 3, 1, 3, 7, and 15 for replications, time, salt, time × salt, error and total, respectively.
[2] Significant at P .01.
[3] Significant at P .025.
[4] Significant at P .25.
[5] Degrees of freedom from hydrogen sulfide, lamb, and ammonia reduced by 6 and compensate for missing values.

selected members. Meat samples generally received a higher rating by the panel if warmed, but the relative ratings remained very nearly the same. Meat products and steam distillates of some of the products were scored by the hedonic scale method of Peryam and Girardot *(15)*. Some canned meat samples had a distinctive off-flavor which was described as a "canned-meat flavor." A canned beef hamburger, not prepared by us, having a very high canned-meat flavor was used as a reference for purposes of comparison. In addition to the general flavor ratings, meat samples were evaluated on a scale divided into 9 categories of intensity of the so-called "canned-meat flavor" ranging from 1 representing *dislike extremely* to 9 indicating *like extremely* or *no canned-meat flavor*. The mean of all of the taste panel ratings for each product is listed as the mean food acceptance score in Table 1. The dividing line between like and dislike is a score of 5 with values below 5 indicating dislike. Some samples receiving slightly lower scores than 5 would have received scores above 5 if the samples had been warmed before tasting; but since it was very difficult to maintain all samples at a constant elevated temperature, samples were tasted at room temperature.

It should be recognized that there are wide variations in eating habits which influence likes and dislikes for foods. It was generally agreed that canned meat products were less acceptable than meat cooked in an open vessel. Some samples receiving low mean food acceptance scores appeared to have received intensive heat processing which resulted in slightly higher than average values for ammonia or hydrogen sulfide and high ratings (low mean food acceptance scores) in *canned-meat flavor*."

DISCUSSION

In general, as the length of the heat processing period increased in canned meat, there was an increase in the concentration of hydrogen sulfide and ammonia in steam distillates of the products.

Steam distillates of the meat products which were given high food acceptance scores by the taste panel generally contained relatively low concentrations of hydrogen sulfide and ammonia. The desirable flavor of cooked meat appears to be associated with low concentrations of volatile nitrogen and sulfur compounds.

The undesirable flavor found in some canned-meat products appears to be associated with high values of hydrogen sulfide and ammonia in steam distillates of these products. Whether the undesirable flavor is directly

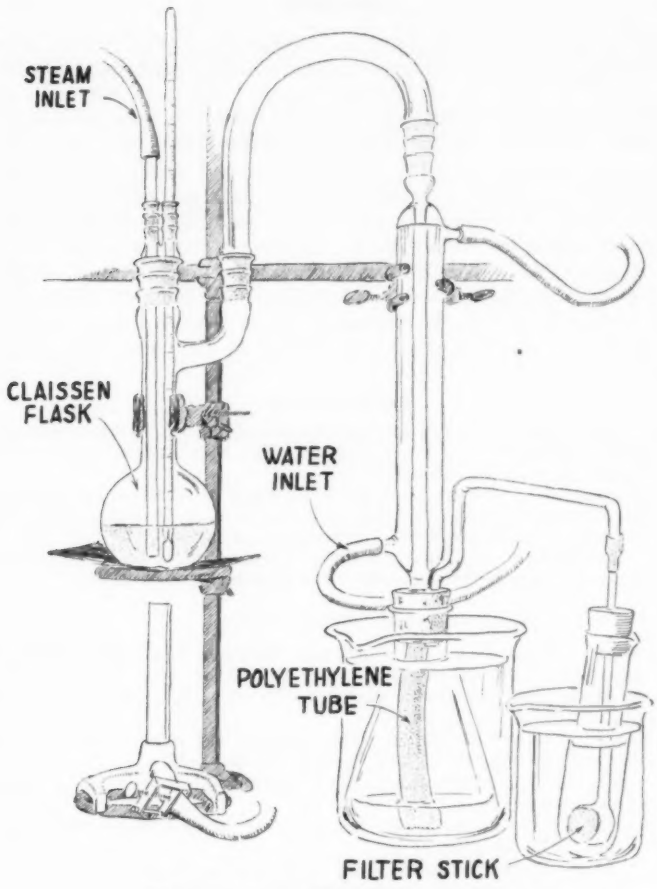

Figure 1. Steam distillation apparatus.

related to the presence of these compounds or to some other compound not yet identified or to chemical changes in the meat during processing is not known.

Mixed food products containing meat and vegetables and cereal give steam distillates which frequently possessed a flavor or odor of the non-meat components as well as meat and generally contained less hydrogen sulfide and ammonia than meat products. Food acceptance responses to mixed food products appeared to be more variable than to a single meat product.

It appears that determinations of the concentration of volatile sulfur and nitrogen compounds in steam distillates of canned-meat products might be used in the evaluation of such products.

SUMMARY

Cooked meat flavors are complex and appear to consist more of odors than of taste. Some of the flavor components of cooked meat may be separated from the meat by distillation (steam) and extraction with certain solvents, e.g., methanol. Steam distillates of fresh and heat processed beef, lamb, pork, and other canned products contained ammonia and hydrogen sulfide as identifiable products. High mean food acceptance scores were usually associated with low concentrations of hydrogen sulfide and ammonia in the distillates. The concentration of these compounds tended to increase in steam distillates as the length of the heat processing period increased. Steam distillates of canned products containing vegetables or cereals frequently possessed a flavor or odor of the non-meat product. The concentration of hydrogen sulfide and ammonia in steam distillates from many mixed food products did not appear to be related to the mean food acceptance rating.

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"BROWNING" REACTIONS AND THE LOSS OF FREE AMINO ACID AND SUGAR FROM LYOPHILIZED MUSCLE EXTRACTIVES OF FRESH AND CHILL-STORED CODLING *(GADUS CALLARIAS)*

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With the development of the newer methods of fish dehydration *(5)*, yielding products of high initial quality, the problems of deterioration during storage have become increasingly important. One of the most serious is the susceptibility of white fish products to brown discoloration during storage at tropical temperatures. Evidence from work on meat *(16)* has indicated that it is in the "extractive" fraction — rather than protein amino compounds — that the development of the "off" flavour associated with "browning" occurs, and work in this Laboratory has supported this approach to the fish problem.

A body of information is now available on the relations of temperature, pH and humidity to the "browning" of codling muscle extractives *(9, 11)* and the manner in which likely amino *(4, 7, 8, 10)* and sugar *(12, 13)* participants in browning reactions change during the period of chill storage, in the fishing vessel and ashore, which invariably precedes dehydration.

This paper describes a study to ascertain the extent of change in the total and individual amino acid and sugar concentrations in lyophilized muscle extractives from fresh and spoiling fish during a period of storage under near-dry conditions and tropical temperatures. Isolated extractives were used because their "browning" has been of some technological importance in processes where the muscle proteins are subject to syneresis in the early stages of dehydration and the substances of low molecular weight dry and brown rapidly at the surface. Furthermore, by comparison with studies on whole muscle systems, it should be possible to elucidate the role of protein in the browning of products in which syneresis has not occurred.

EXPERIMENTAL

Codling, 18-24 inches in length, were trawl-caught. Fish for fresh controls were landed alive and maintained in sea-water for under 24 hours until killed for immediate analysis. Other fish were eviscerated and packed in ice at an ambient 2.5° C., simulating conditions in a commercial trawler. Batches were removed for analysis periodically. It was not practicable to follow, throughout a storage period, changes in a single fish. The removal of samples of muscle for analysis would expose fresh surfaces to bacterial attack and leaching action by melt water so that conditions become atypical of commercial storage in which the muscle remains intact.

Preparation of extracts. The fish were filleted and 20 g. of muscle, dissected from the anterio-dorsal portion, were extracted into 250 ml. of 75% (v/v) ethanol. To a 25 ml. aliquot were added 75 ml. of chloroform at 15° C. The upper, aqueous layer, which separated from the emulsion, contained the non-protein, water-soluble compounds of the muscle *(1, 9, 12)*.

"Browning" of extracts. Aliquots (2 ml.) of the chloroform-separated, ethanolic extracts were lyophilized in shallow, wide-necked bottles and equilibrated in air to 60% Relative Humidity and 40° C. over a sulphuric acid-water mixture. The bottles were

maintained under these conditions for 3 days and the volume was then adjusted to 2 ml. for the measurement of brown colouration at an arbitrary 430 mμ. in a Hilger "Spekker" photoelectric absorptiometer microcell attachment. Where necessary "browning" stages were replicated to obtain enough material for subsequent analysis.

Estimation of free amino acid. Aliquots (2 ml.) of unbrowned, original, chloroform-separated, ethanolic extract were lyophilized and stored at −2° C. for analysis. The "browned" preparations were re-lyophilized after the measurement of colour and stored similarly. Both "browned" and untreated extractives were dissolved in 300 μl. of distilled water at 0°.

An aliquot, predetermined by rough experiment to fall within a 3-12 μg. amino acid standard range, was placed in successive 0.2 μl. droplets at a corner of an 8 inch square of Whatman No. 1 filter paper in a stream of chilled air. Estimations were triplicated and batches of sheets and standards were placed on a frame *(2)* for two-dimensional chromatography in phenol-ammonia and collidine-lutidine *(3)*. The papers were air-dried after treatment with each solvent and sprayed on both sides with 1% (w/v) ninhydrin in water-saturated butanol. Colour was developed at 15° C. in darkness over 2 days. Spots were cut out and eluted into 2 ml. of 50% (v/v) aqueous acetone, blanks of equivalent, adjacent area being similarly treated. The extracted colour was measured at 570 mμ. as described above. By reference to the standard curves, individual amino acids were estimated with an accuracy of ± 7% at worst and of ± 4% with the best separations.

Estimation of free sugars. Aliquots of redissolved extractives, as prepared for amino acid estimation, were chromatographed on Whatman No. 1 paper in butanol-acetic acid *(14)*, without pre-treatment as described previously *(12)* but with chilling during the application of the extractives to the paper. The dried papers were sprayed with aniline hydrogen phthalate solution *(15)*, heated at 105° C. for 5 minutes and the spots were cut out, eluted into acetic acid and evaluated at 480 mμ. as above. The precision was ± 6%.

RESULTS

The browning of lyophilized extractives. After 3 days at 40° C. and 60% R. H. the extractives from the flesh of both freshly killed and chill-stored codling had all developed brown colouration to varying degrees (Fig. 1). Absorptions at 430 mμ

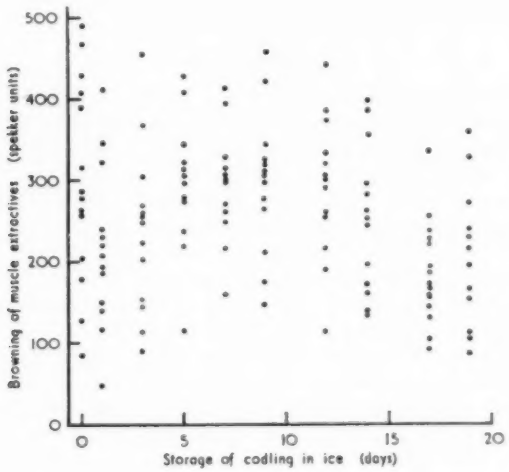

Figure 1. "Browning at 60% R.H., 40° C. of lyophilized muscle extractives from codling stored in ice.

ranged widely, particularly in the fresh samples and those stored from 1 to 3 days in ice. There was a slight tendency for "browning" values to rise slightly in extractives from fish stored in ice from 5 to 12 days and to be less scattered. Thereafter, values fell but throughout the whole period of storage in ice variations were small compared with those within each group.

Free amino acid contents and their losses at 60% R.H., 40° C. With the exception of 1-methylhistidine, changes in which have been reported elsewhere *(11)*, the major amino acids were determined in a proportion of the samples. In agreement with previous findings *(8)* taurine was the principal constituent of the fraction in fresh muscle, exceeding the total of the other amino acids (Table 1.). During storage in ice the taurine levels tended to fall, β-alanine to rise and the levels of glutamic acid, leucine and α-alanine to remain roughly constant (cf. 17). Lysine levels in fresh material were high in relation to the yearly average and taurine lower. (This is characteristic of fish caught in the October-December period). Small seasonal departures from average trends were also noted during chill storage in relation to glycine levels, which did not rise at the usual rate after the customary initial fall, and in lysine, which also failed to maintain an average rate of increase throughout storage *(17)*.

Reaction at 40° and 60% R. H. in air led to losses in all of the amino acids examined from extractives of fish at all stages of chill-storage. The losses were usually of the order of 40-60% irrespective of the initial concentration or of the amino acid. Occasionally, they were considerably higher, as in lysine from fish iced for 24 hours. In no case was it possible to attribute a high level of browning to the initial concentration, or loss, of any particular amino acid under these conditions.

Free sugar contents and their losses at 60% R.H., 40° C. The free sugars present in codling muscle are glucose and ribose. In the present experiments, the glucose values in fresh muscle varied considerably, as described previously *(13)*. During a period of storage in ice this sugar tended to disappear from the muscle and ribose to increase initially from zero levels and then fall after 10 to 12 days' storage. Ribose values were low in comparison with previous findings; this point is considered later.

During the storage of extractives at 60% R. H. and 40° C. (Table 2) for 3 days, ribose disappeared entirely and occasional traces only of glucose remained.

In extractives from fish stored for 0 to 3 days in ice, the amount of "browning" was roughly related although not directly proportional to the glucose content of the muscle. From 12 days storage in ice onwards "browning" was related to the ribose concentration (glucose values being very low). The intermediate picture was less clear but presumably was the result of a summation effect.

DISCUSSION

The chromatographic procedures reported in the present paper for amino acid and sugar analyses are of a type commonly used in such studies and it may be of some value to point out their limitation. Firstly, there is a possibility of reaction during the application of extracts to the start-line of paper chromatograms *(12)*. Such considerations are of little importance in the application of extractives already subjected to 40° C. and 60% R.H. but they could be so during the analysis of controls. While initial reactions on a 1:1 molar basis with the very limited quantities of muscle sugar would be unlikely to effect considerable changes in amino acid concentrations, those of the sugars — particularly ribose — are susceptible. In the present experiments, great care was taken to minimise such reactions by drying at low temperatures, but, by comparison with values in extractives treated with ion-exchange resins *(12)* to remove reactive amino compounds, concentrations of ribose were some 20% low: glucose values were not affected. Nevertheless, the procedure was followed because batchwise or column treatment with resins after reaction is likely to lead to greater complications (cf. 12) such as the hydrolysis of reaction intermediates, particularly those of the early stages of "browning" reaction-chains. The same considerations also apply

TABLE 1

Free amino acids in extractives from fresh and chill-stored codling muscle before and after "browning" at 60% R.H., 40°C.
(Amino acids are expressed as mg. per 100 g. original tissue; figures are averages of five fish with ranges in parenthesis. "Browning" is expressed as "Spekker" units (see text)).

Days of storage in ice	Glutamic acid		Glycine		Taurine		α-Alanine		β-Alanine		Lysine		Leucine		"Browning"
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	
0	9.1 (7.2-12)	4.8 (3.8-5.9)	17 (14-19)	8 (7-10)	200 (170-250)	96 (79-114)	23 (20-29)	8 (5-13)	2.9 (1.6-6.4)	1.1 (0.5-2.3)	10 (7-13)	5 (4-7)	10 (6-16)	4 (2-7)	272 (177-403)
1	6.6 (5.2-7.8)	3.1 (2.1-3.8)	6.8 (5.2-8.4)	3.5 (2.6-5.1)	150 (130-180)	66 (59-72)	18 (15-24)	8 (5-12)	7.4 (5.7-8.4)	3.0 (1.8-4.1)	26 (15-33)	6 (4-8)	5.5 (3.8-8.1)	2.6 (1.6-3.7)	236 (113-346)
3	9.3 (6.8-11)	4.2 (2.8-5.2)	10 (8-16)	5 (4-6)	120 (100-130)	58 (49-66)	22 (18-28)	10 (8-12)	13 (9-15)	6 (4-8)	28 (26-33)	8 (5-14)	3.8 (2.8-5.1)	1.6 (1.0-2.0)	214 (179-319)
5	8.0 (5.5-11)	3.9 (2.7-5.1)	8.4 (4.5-11.2)	2.9 (1.2-4.8)	110 (81-130)	44 (37-52)	18 (12-23)	8 (5-12)	15 (12-18)	5 (4-6)	28 (21-38)	9 (6-12)	3.8 (2.7-6.1)	1.3 (0.9-1.9)	304 (220-429)
7	9.8 (7.5-13)	5.0 (4.1-6.3)	7.7 (3.9-10)	5.4 (3.6-6.4)	82 (71-92)	41 (31-52)	16 (14-18)	9 (6-14)	22 (19-32)	8 (7-10)	24 (20-28)	8 (7-10)	3.2 (2.3-4.8)	1.7 (1.0-2.5)	275 (158-413)
9	9.4 (5.6-15)	4.7 (2.9-8.1)	9.9 (7.9-11)	5.0 (3.8-5.8)	69 (51-81)	36 (29-40)	15 (11-18)	8 (6-10)	27 (21-30)	12 (11-15)	15 (10-20)	7 (6-9)	4.4 (2.8-7.5)	1.9 (1.6-2.4)	293 (172-419)
12	8.7 (7.3-12)	4.4 (3.0-6.0)	5.8 (4.2-8.3)	3.3 (2.0-4.4)	65 (58-72)	40 (30-54)	16 (13-19)	6 (5-6)	30 (24-33)	11 (7-17)	15 (10-21)	5 (3-8)	4.4 (3.1-6.0)	2.1 (1.4-2.9)	302 (190-441)
14	7.1 (6.1-11)	4.6 (2.8-6.0)	6.8 (4.2-8.9)	4.0 (3.7-5.1)	61 (49-73)	31 (23-36)	18 (13-23)	7 (6-8)	24 (19-31)	10 (8-15)	22 (11-27)	10 (5-14)	4.7 (2.6-8.2)	2.1 (1.4-2.9)	207 137-281)
17	7.5 (3.9-12)	3.7 (1.9-5.4)	7.2 (3.9-10)	3.5 (1.8-4.8)	57 (41-78)	25 (21-29)	11 (8-13)	5 (4-6)	21 (11-29)	10 (5-13)	18 (12-25)	8 (6-11)	4.9 (2.9-6.7)	1.7 (2.0-3.7)	177 102-340)
19	6.4 (3.6-8.2)	4.1 (3.3-4.5)	6.7 (2.7-10)	3.0 (1.9-4.6)	51 (30-72)	23 (17-26)	10 (7-14)	8 (4-13)	20 (12-28)	8 (4-13)	14 (9-18)	5 (0.8-9)	5.5 (3.4-7.3)	2.3 (1.6-3.0)	197 (104-357)

TABLE 2

Free sugars in extractives from fresh and chill-stored codling muscle before and after "browning" at 60% R.H., 40°C.

(Sugars are expressed in mg. per 100 g. original tissue; "browning" expressed as "Spekker" units (see text)

Days of storage in ice	Fish No.	Ribose		Glucose		"Browning"
		Before	After	Before	After	
	1	0	0	22	0.24	262
	2	0	0	31	0.31	391
0	3	0	0	11	0.20	126
	4	0	0	15	0.30	177
	5	0	0	33.2	0.36	403
	6	0	0	19	0.24	204
	7	0	0	9.2	0	113
1	8	0.11	0	30	0.52	346
	9	0	0	17	0.21	194
	10	0	0	29	0	321
	11	0.20	0	12	0	179
	12	0	0	11	0.25	143
3	13	0	0	29	0.31	319
	14	0.14	0	15	0	205
	15	0	0	20	0.24	223
	16	0.18	0	19	0	235
	17	0.52	0	15	0	220
5	18	0.31	0	29	0.35	340
	19	1.1	0	13	0	296
	20	0.84	0	32	0.49	429
	21	1.3	0	23	0.54	325
	22	0.51	0	16	0	218
7	23	4.0	0	9.6	0	413
	24	1.2	0	18	0.21	262
	25	1.5	0	11	0	158
	26	2.5	0	5.4	0	172
	27	3.1	0	11	0	261
9	28	3.8	0	10	0	302
	29	1.4	0	18	0.37	310
	30	4.6	0	3.1	0	419
	31	3.6	0	8.2	0.21	384
	32	4.4	0	10	0.41	441
12	33	2.8	0	1.8	0	290
	34	1.0	0	2.6	0	214
	35	0.9	0	5.8	0.22	190
	36	1.8	0	0	0	167
	37	2.4	0	2.4	0.38	259
14	38	0.61	0	1.6	0	137
	39	3.1	0	0	0	281
	40	1.9	0	0	0	193
	41	1.8	0	0.81	0	173
	42	0.51	0	0.72	0.21	102
17	43	1.4	0	0	0	144
	44	4.1	0	0	0	340
	45	0.69	0	0.41	0	127
	46	1.7	0	0	0	162
	47	0.41	0	0	0	104
19	48	4.2	0	0	0	357
	49	0.85	0	0	0	153
	50	2.1	0	0	0	211

Zero values are < 0.2 (glucose) and < 0.1 (ribose)

to the separation of "browning" reaction intermediates on ion-exchange resin columns; there is a considerable danger of artefact production. To some extent this is also a problem with contact with the acid and alkaline solvents used in paper chromatography but there is not the same intimate binding to conjugated systems which are known to cause changes in compounds containing sugar residues.

While bearing in mind that the experiment necessarily lacks the accuracy that one would wish for in ribose analysis, it is possible to arrive at certain conclusions:

The study demonstrates that there is little difference in the average "browning" potential of muscle extractives from codling at different stages of spoilage under commercial chill conditions. Variations between individual fish at the same stage are considerable, particularly during the 0-3 day period.

The conditions under which the "browning" was carried out favour sugar-amino reaction at the expense of 1-methylhistidine degradation, a potent mechanism in the discolouration of codling muscle in the absence of reactive sugar. This mechanism has been reported elsewhere *(11)* and the behaviour of the amino acid was not followed in the present experiments.

The data presented indicate that glucose is the limiting factor in the "browning" of extractives from fish in the very early stages of deterioation during chill storage and that ribose becomes important later. As the reaction-sequence is very complicated, direct proportionality was not to be expected and was not found between sugar concentration and "browning." The concentrations of the two sugars present in the muscle are such that the greater reactivity of the pentose in spoiling muscle appears to be counter-balanced by the greater quantity of glucose present in fresh material, although the effects of any sugar phosphate remain to be elucidated. The net effect is that there is little difference in the "browning" potentials of codling after different periods of chill storage. Towards the end of the storage period in ice, when the browning potential does fall a little, the fish is unsuitable for dehydration for other reasons.

The extent to which amino acid is lost from extractive mixtures is considerably in excess over that to be expected from 1:1 reaction with sugar (and the limited amounts of sugar phosphate present in the muscle). This is not unexpected in relation to Hodge's scheme *(6)* of the Amadori rearrangement in the integration of known reactions leading to "browning" in sugar-amine systems. Both the 1-amino-1-deoxy-2-ketose (1,2 enol form), produced by rearrangement of the postulated initial n-substituted glycosylamine, and compounds resulting from the removal of the elements of water from 1-amino-1-deoxy-2-ketoses can react with further amino groupings in complex reaction chains. At the same time, the data does indicate that the sugar concentration and the type of sugar in the muscle are limiting factors in the "browning" reactions of extractives.

Further work is in progress on the kinetics of "browning" in model, phosphate-buffered, sugar-amino systems with particular reference to the individual compounds present in codling muscle extractives.

SUMMARY

Muscle extractives, from codling stored in ice for different periods of time, have been maintained at 40° C and 60% R.H. for 3 days.

The amount of "browning" resulting from this treatment varied considerably in extractives from different fish stored in ice for the same length of time: variations between batches were small in comparison. "Browning" was accompanied by the disappearance of the limited quantities of sugar in the extractives and losses in amino acid greater than would be expected by 1:1 reaction with sugar. Glucose was the limiting factor in the "browning" of extractives from fresh codling muscle and ribose in those from spoiling muscle.

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QUALITY OF PORK IN RELATION TO RATE OF pH CHANGE POST MORTEM

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In recent years there has been a growing interest in studies of the physiological and biochemical factors of importance to the colour and water holding capacity of meat. A part of this increasing interest is undoubtedly caused by the fact that to be competitive meat products primarily require quality. Thus, the market for fresh meat becomes more and more discriminative.

Pork seems particularly variable from animal to animal in colour and water holding capacity and therefore deserves special interest. Existence of considerable variation in these aspects of pork quality has been known for many years. Pale and watery pork appears, for example, to have caused German sausage makers considerable trouble more than 50 years ago *(16)*. The tendency to produce watery pork does not appear to be confined to any particular breed. Under identical conditions, there may, however, be differences between breeds in the degree of water imbibition of the pork *(19)*. In the veterinary literature studies on pigs have been reported in which the meat has been observed to be gray and watery after normal slaughter or on autopsy after instant death on the farm *(23–26, 14, 32, 27)*. Excessive feeding of potatoes *(18)* or hereditary disposition in connection with stress *(26, 15)* have been offered in explanation.

In this paper some observations on differences in structure, as characterized in the water holding capacity, and colour of pork will be given. The meat under study came exclusively from Danish landrace pigs of 180-200 lbs., live weight. The pigs grew and otherwise behaved in an entirely normal manner. The meat was within the quality range that pork usually has when offered for consumption in Denmark. Very rarely are cases of real "muskeldegeneration", as described by Ludvigsen, encountered. The pigs were reared at the progeny testing station "Sjaelland" and slaughtered at Roskilde bacon factory after electrical stunning. The studies have been concentrated on the loin *(longissimus dorsi)* a muscle, which in common experience is one of the most variable in colour and texture. The colour of this muscle has therefore been selected to represent the meat colour in the carcass evaluation as it forms a part of the progeny testing of the pigs *(9)*.

Meat structure. Callow *(5, 6, 7, 8)* was probably the first to study the structure of pork from a technological point of view. He found that the meat could have either an open structure characterized by low electrical resistance and moist appearance on cutting, or a close structure characterized by considerable electrical resistance and dry appearance on cutting. The difference in structure depended on pH and the rate of cooling when the carcass was chilled after slaughter.

In this study attention has been given to the effect pH and meat temperature may have on the structure. This appears especially appropriate since Ludvigsen *(23)* has noticed that the pale and watery meat from pigs with "muskeldegeneration" had a pH of about 5.4 when measured 30 minutes after slaughter. A corresponding acidity in the meat at a time when the full body heat still remains may conceiverably be of importance

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to the structure of the meat when ready for use. It is well-known that even mild temperatures have a negative effect on the water imbibition of meat *(20)*. To establish whether similar low pH values might be encountered in the pigs under study the pH was measured in the loin ¾ hour after sticking, i.e. as soon as it is possible under practical conditions in the abattoir. The day after slaughter a sample was cut out of the loin at the point where pH was measured to test the water holding capacity. At the same time a new measurement of pH was taken.

EXPERIMENTAL

Analytical methods. *The pH* was measured by direct insertion of the electrodes in the loin right at the back rib. Radiometer pH meter model 24 equipped with glass electrode G213 and kalomel electrode K4011 was used. The results of the direct measurements were occasionally checked with pH measurements in samples macerated in 0.005 M sodium iodoacetate.

Water holding capacity was tested by use of a modification of Grau and Hamm's press method *(12)*. A pea size, (0.7 g.) meat sample was cut out in the middle of the loin. The sample was immediately pressed between two filter papers with standardized water content. The filter paper was Struers' standard quality kept in a dessicator with saturated KCl solution. The pressing took place in a plexiglass compressorium, the plates of which were screwed firmly together for 5 minutes. After pressing, the meat was separated from the filter paper and weighed. The weight loss compared to the initial weight of the sample was used to denote the water holding capacity. The weight in mg. per g. meat will be termed the LW number, loose water number. A high number thus denotes a low water holding capacity. All the expressed LW numbers are averages of 2 determinations.

Solubility in 0.6 M KCl: Twelve and a half grams of meat was mixed in 50 ml. cold 0.6 M KCl with an Ultra Tarrax mixer for 1 minute. After 5 minutes 150 ml. cold 0.6 M KCl was added to the mixture, which was remixed for ½ minute. The mixture was centrifuged for 20 minutes at 1500 g. With the precipitate the whole operation was repeated. The weight of the final precipitate was recorded, the nitrogen content was determined after Kjeldahl, and the percentage of nitrogenious material of the meat insoluble in the salt solution was recorded.

Glycogen content was determined after boiling with potassium hydroxide, precipitation and hydrolysis *(4)* as reducing sugar.

Lactic acid and reducing sugars: The contents were determined on a trichloroacetic acid extract of the meat. The lactic acid content was determined after Barker and Summerson *(1)*.

ATPase activity of the meat was estimated essentially as done by Dickens & Salmony *(11)* on myosin. Instead of myosin a quantity of homogenized, washed meat, devoid of tendons and fatty material was used. The quantity corresponded to 1 mg. dry matter.

RESULTS AND DISCUSSION

Water holding capacity in relations to initial pH

In Figure 1 the water holding capacity of the meat tested the day after slaughter is related to the pH as measured ¾ hour after sticking; pH measured at that time will hereafter be termed pH_1. Each point on the figure denotes an individual loin. One notices that the pH measurements are spread over a range from about 5.6 to about 6.6. If all observations are taken together one sees a general decrease in water holding capacity with decreasing pH. The correlation coefficient btween LW and pH_1 was calculated to —0.71, which is statistically significant at $P = 0.001$.

For the loins with pH_1 above 6.2 the LW-values are predominantly below 460; the values for the loins with pH_1 below 6.2 are predominantly above 460. The corresponding correlation coefficients between pH_1 and LW for the loins with pH_1 above and below 6.2 are —0.38 and —0.13, respectively.

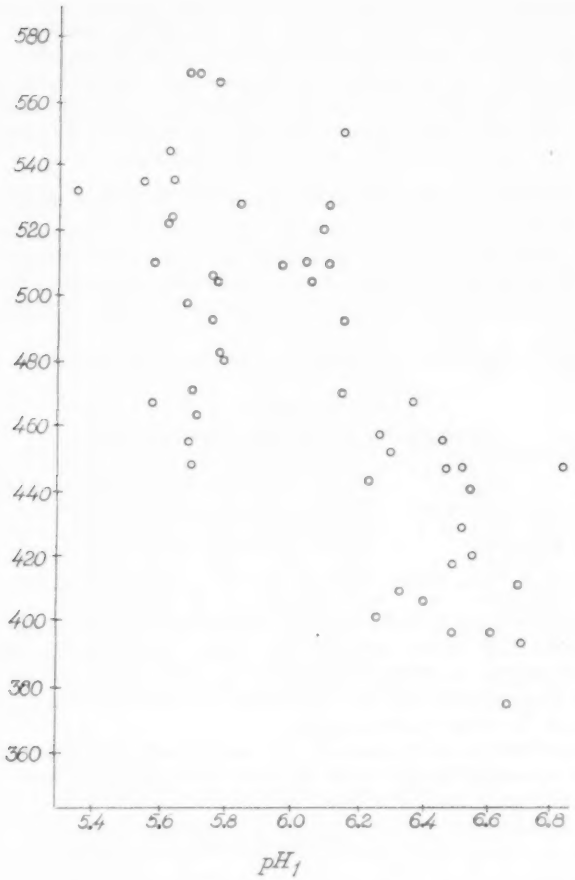

Figure 1. LW number measured approximately 20 hours after slaughter.

The last mentioned coefficient is far from significant, which means that pH_1 differences in the low pH range have only slight effect on the water holding capacity. The first mentioned coefficient has a statistical probability of significance between $P = 0.10$ and $P = 0.05$; hence, we may here encounter some relationship between LW and pH_1.

The pH of the same loins, recorded at the time of the LW measurement, was in the range between about 5.3 and about 5.6. The coefficient of correlation between LW and pH was calculated to —0.20 which is far from statistical significance.

The ultimate pH of the meat in this study is so uniform that the well-known effect of the ultimate pH on the water binding capacity *(13)* is precluded.

Simultaneous measurements of pH_1 in the loin and gammon *(adductor)* was done in a preliminary experiment. The correlation coefficient between the two muscles was +0.543 and found to be statistically significant at P = 0.001. The results of the studies of the loin may therefore be applicable to the gammon musculature. A similar relationship was found between pH_1 in the loin and tenderloin *(psoas)*. Between the pigs the variation in pH_1 was greater for the loin than for the two other muscles.

Chemical changes in the meat with low water holding capacities

The differences in the LW numbers might either be due to differences in the water content of the meat or to differences in the water holding capacity. To check these possibilities meat samples taken with the samples for the LW test were analysed for dry matter, mineral matter, and nitrogen content after Kjeldahl.

In Table 1 results for 10 samples are given. The results are averaged

TABLE 1
Composition of the meat in relation to LW

LW	% Water	% Mineral matter	N-free extr.	% Total N	% of total N insoluble in 0.6 M KCl
500-540	73.2	1.14	1.8	3.83	50
460-499	71.5	1.15	2.4	3.98	33
420-459	71.6	1.13	3.2	3.85	24

in groups according to the LW numbers. No real difference is apparent with regard to the LW values although the water content in the group with highest LW number is slightly higher than the water content in the two other groups. It is concluded that the differences in the LW values are due to differences in the water holding capacity.

This conclusion is in the line with one arrived at by Professor Meinike in Wiesbaden around 1908 *(16)*. He analysed two samples of ham meat. One sample was from a ham with meat of nice colour and firm structure and the other from a ham with pale, watery meat. He found that the water content in the watery meat was only slightly higher than in the meat with the firm structure. From these results the Germans concluded that the watery meat differed less in composition than in structure from the desirable meat. They suggested that cells in the watery meat had a diminished ability to retain the meat juice.

The differences in water holding capacity might be due to partial denaturation of the meat proteins, in particular actomyosin. Denaturation of the actomyosin should manifest itself in decreased solubility in 0.6 M KCl and loss of its enzymatic activity, i.e. its ATPase activity.

Table 1 includes the percentage of the total nitrogen content of the meat which is insoluble in 0.6 M KCl. One notices that on the average 24% is insoluble in the group with LW number below 460. For the meat with LW numbers between 460 and 500 the percentage has risen to an average of 33% and in the group with LW numbers above 500 to 50%. It appears that the decreased water holding capacity of the meat is connected to an alteration of the meat proteins which has made them partially insoluble in 0.6 M KCl.

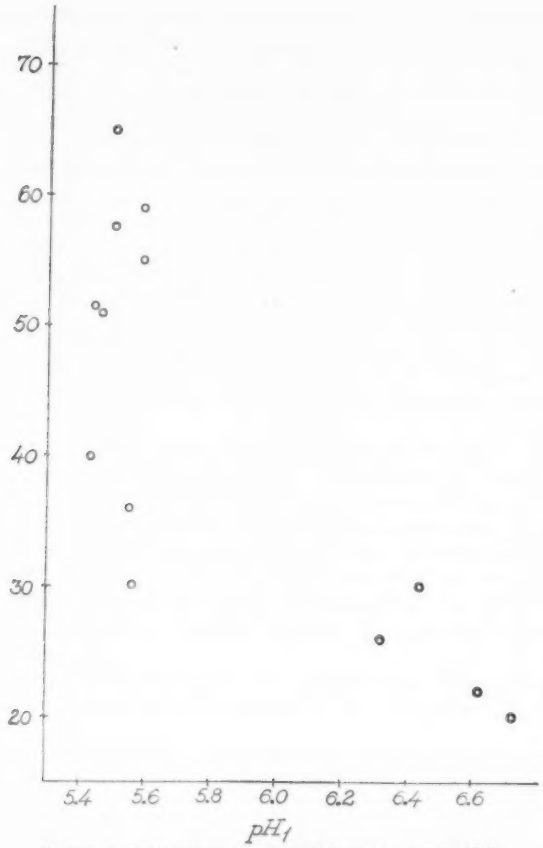

Figure 2. Percentage of meat N insol. in 0.6 M KCl.

In Figure 2 the solubility measurements of 13 similar samples are shown in relation to the corresponding pH_1. One notices that the low percentages of insoluble material are connected to pH_1 above 6.2. When pH_1 is low the solubility is very variable between the samples of approx. identical pH_1 values. Similar variation was noticed for LW numbers (Figure 1).

The measurement of the ATPase activity of samples of loins the day after slaughter did not reveal any close relationship between the enzymatic activity and LW or pH_1. Low ATPase activity was, however, predominantly associated with pH_1 values below 6.

Low water holding capacity of the loins appears to be associated with structural alterations in the protein towards decreased solubility in 0.6 M KCl. However, at times, the ATPase activity was also diminished. The impact of the low pH_1, or the processes which give rise to it, result first in decreased solubility of the proteins (and increased LW number) and then secondly in diminished ATPase activity.

Decreased solubility and increased LW number has been demonstrated by heating a sample of a loin with normal pH_1 and LW number in a closed petri dish at 32-33° C. for 2 hours. The heating took place the day after slaughter when the pH of the meat had fallen to 5.45. The LW number went from 458 to 546 and the insoluble matter increased from 16 to 69%.

Continuous record of pH and temperature in the loin

In Figure 3 examples are given of the pH and temperature variations

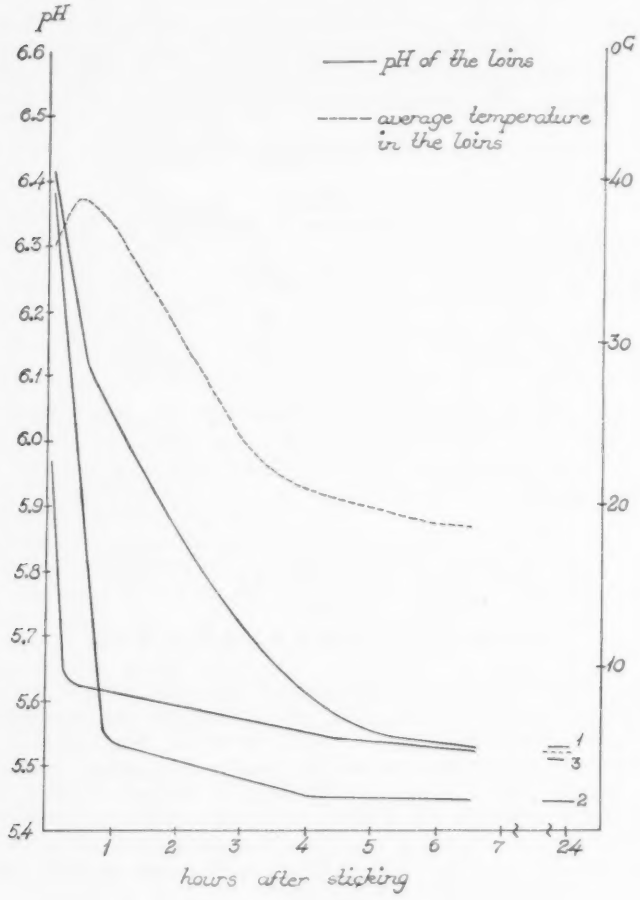

Figure 3.

which may take place in the loin beginning a few minutes after sticking. After the slaughtering operations the carcasses were kept under hanging floor conditions. The measurements were taken on the carcasses from 3

barrows reared on the experimental farm "Sjaelland II" in exactly the same manner as on the progeny testing stations. The pigs were slaughtered at the Roskilde bacon factory before the normal slaughtering of the day took place. It was thus possible to do the measurements between normal slaughtering processes like scalding, singeing, etc. The measurements were taken in the loin at the back rib. The measurements continued 6½ hours after slaughter. After the measurements the sides were placed in a chilling room. The next day the loins were cut where the measurements took place and samples taken for measurement of LW number and ATPase activity.

For pig 1 the LW number was 421. The corresponding pH fall (1 on Fig. 3) may therefore be regarded as harmless to the meat structure. The colour of the cut surface of the loin was judged excellent (score 2½ on the scale p. 725). For pig 2 and 3 the LW numbers were 482 and 494 respectively. The corresponding pH falls (2 and 3 on Fig. 3) may therefore be regarded harmful to the meat structure, 3 more so than 2. The meat colour was judged pale and the meat had a wet appearance (score 1 and ½ respectively).

The ATPase activities were 15.3, 14.3, and 9.7 for pig 1, 2, and 3 respectively. The units of activity is μg. P liberated on incubation in 15 minutes at 30° C. It appears that the pH drop corresponding to 3 has caused changes in the meat proteins and hence the enzymatic activity has decreased. For pig 2 the fall in pH has decreased the water holding capacity but insignificantly depressed the ATPase activity compared to the measurements for pig 1. This result is in line with the notion that the differences in pH variations affect the water holding capacity to a greater extent than the enzymatic activity.

In order to get a starting point for consideration of the pH drop in the carcass an attempt was made to measure the pH while the pig was still alive.

About a fortnight before slaughter the barrows were subjected to trichlorethylene anaesthesia and a sample of the loin muscle removed. The sample was instantanously macerated in iodoacetate and pH measured. The pH was 6.75, 6.69, and 6.67 for pigs 1, 2, and 3, respectively. All pH's were thus relatively high and reflect strikingly the drastic pH changes which take place in the course of slaughter. Under the stress which the anaesthesia and biopsi undoubtedly imposed on the animals the pigs 2 and 3 show lower pH values compared to pig 1. When this observation is related to the drop in pH after slaughter it appears likely that the initial step of the fall in pH at slaughter starts before death, under influence of the stress to which the animal is subjected.

THE CAUSE OF THE DIFFERENCE IN RATE OF pH FALL

Analysis of the meat

The rapid fall in pH which gives rise to the low pH_1 values appears due to formation of lactic acid. The content of lactic acid was determined in samples from 47 loins with pH_1 ranging from 5.6 to 6.7. The samples were taken when pH_1 was measured and frozen with carbon dioxide snow until determination could take place. The coefficient of correlation between pH_1 and the corresponding concentration of lactic acid was worked out to be -0.86 and found to be statistically significant at $P = 0.001$.

In Table 2, results of analysis of the content of glycogen, reducing sugars, and lactic

TABLE 2
Content of glycolytic compounds in meat with different pH_1

Samples taken ¼ hour after slaughter				Samples taken 20 hours after slaughter				
pH_1	Glyogen	Reducing sugars	Lactic acid	pH	Glyogen	Reducing sugars	Lactic acid	LW
5.68	0.27	0.61	1.03	5.20	0.07	0.96	1.42	534
5.78	0.32	0.78	1.04	5.41	0.08	1.20	1.35	514
6.08	0.37	0.41	0.77	5.38	0.17	0.56	1.28	502
6.24	0.56	0.51	0.61	5.27	0.14	0.81	1.21	462
6.44	1.17	0.72	0.59	5.32	——	1.34	——	459
6.56	0.83	0.49	0.50	5.27	0.08	0.82	1.20	423
6.76	1.06	0.28	0.34	5.36	0.10	0.59	1.13	415

acid are given for 21 loins. Determinations were made on samples frozen ¼ hour after slaughter and on samples taken 20 hours after slaughter. Results are averaged in 7 groups with increasing pH_1 values. Due to the relationship between LW values and pH_1 the groups are also arranged according to decreasing LW values. It is apparent that the rapid formation of lactic acid is due to an accelerated glygolysis. The table also illustrates that the LW values are independent of the pH values at the time of LW measurement, as mentioned before, although the samples with high LW numbers appear to have slightly higher lactic acid content.

The content of total, inorganic and "7 minutes' phosphate" in the meat was estimated in the above mentioned samples. "7 minutes' phosphate" is usually used as an estimate of the ATP content. The methods of analysis were as given by Bate-Smith and Bendall *(2)*. The results did not reveal any differences of interest in the interpretation of pH_1. The content of P_7 min. was on an average 0.21 mg./g. meat when pH_1 was below 6.2 and 0.25 mg./g. meat when pH_1 was above 6.2. The results suggest that a violent splitting of ATP takes place in the meat with low pH_1 if one considers that 1½-2 mol. ATP are reformed for each mol. of lactic acid formed *(22)*.

Determination of magnesium and calcium in the meat did not show any difference in concentration in relation to pH_1. Magnesium was determined gravimetrically and calcium titrated with permanganate after precipitation with oxalate. The determinations were carried out on ashed samples of the meat.

The concentration of potassium was on the average a little lower in the meat with low pH_1. There was, however, considerable variation between the loins with similar pH_1; so low pH_1 was not always connected to low potassium content. The method of analysis was essentially as described by Ludvigsen *(23)*. The lower potassium content may be ascribed to leaking of potassium from the cells. The extravasation of potassium was also noted by Ludvigsen *(23)* in his examination of the meat from pigs with "muskeldegeneration". It is probably due to formation of lactic acid in the cells before death *(15)*.

In an attempt to study the mechanism of the accelerated lactic acid formation the phosphorylase activity was estimated in 6 tenderloins *(psoas)* with different pH_1. The estimation was made essentially as described by Sutherland and Cori *(28)*. The meat was macerated as soon as possible after measurement of pH_1. The maceration was done in a cold 0.04 M phosphate buffer, pH 7.4, containing 0.001 M versene and 0.02 M sodium fluoride. The macerate was incubated for 30 minutes at 37° C. with an amount of glycogen corresponding to 1% of the weight of the meat. In Figure 4 the percentage of the added glycogen decomposed during the incubation is shown in relation to the pH_1 of the meat for each of the 6 tenderloins. The results suggest that the phosphorylase activity is increased in the meat with low pH_1. The amount of lactic acid and reducing sugar formed had no quantitative relationship to the decomposed amount of glycogen. The discrepancy is probably due to disorganization of the cell structure on the maceration. The increased phosphorylase could possibly be a consequence of adrenalin released from the adrenals. Estimation of adrenalin content in blood samples taken at the sticking operation failed, however, to show any relation to the pH_1 values of the loins. The method of estimation was essentially as described by Weil-Malherbe and Bone *(30)*.

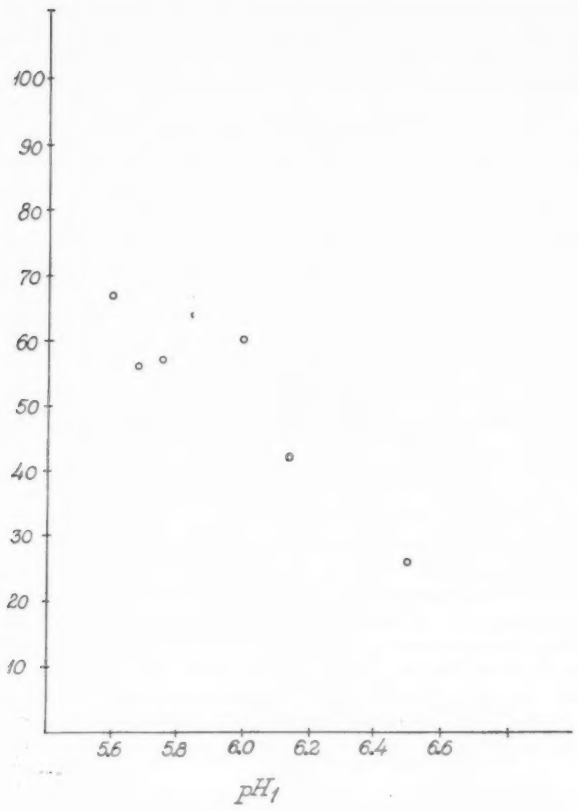

Figure 4. Percentage of added glycogen decomposed during incubation.

Histological examination

Samples of loins with different pH_1 were examined histologically.[b] No systematic difference in the appearance of the muscle fibres and the cell structure has so far been encountered between the meat with high and low pH_1.

Observations on the pigs

The rapid production of lactic acid appears to be a consequence of increased demand of energy in the cell which exceeds what can be achieved through the aerobic metabolism. This demand may be a further consequence of a stress on the animal. The inference that stress may be involved is in line with common experience in the trade that more pigs with pale and watery meat show up when the pigs have a strenuous transport to the

[b] By Erna Christensen, M. D., Rigshospitalet, to whom my thanks are due.

abattoir, especially in hot weather. It may also be shown that the amount of fighting and turmoil among the pigs in the pens before slaughter has a pronounced influence on the occurrence of meat with low pH_1.

In Figure 5 observations on the behaviour of pigs in the pens at the abattoir

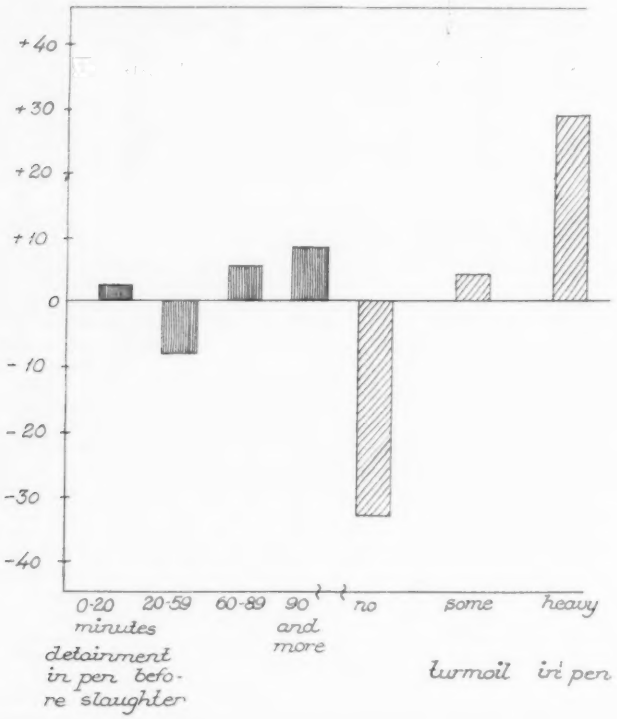

Figure 5. Percentage increase or decrease of loins with $pH_1 < 6.2$.

before slaughter are related to variations in the frequency of pigs with loins having pH_1 below 6.2. The figure also shows the frequency in relation to the time the pigs are detained in the pens. The pigs under observation were 850 pigs from the progeny testing stations delivered to the abattoirs in the months June, July and August 1957 after less than 13 km. transport by lorry. The pigs were delivered to the abattoirs in lots of 20-30 pigs each week. The lots were watched for the length of time in the pen before slaughter and the degree of turmoil. The lots were then classified in accordance to turmoil and detainment and the percentage of pigs giving $pH_1 < 6.2$ were averaged among the pigs noted. The decrease or increase in occurrence of low pH_1 in percentage of the average frequency is given below and above the O line. When the pigs were slaughtered within 20 minutes after delivery the frequency was 2% higher than average. If there were allowed a detain-

ment of 20-59 minutes before slaughter the frequency was 7% below average. For longer detainments the frequency rose above the average as the pigs would begin fighting. Fighting obviously has considerable influence on the frequency. If the pigs appeared calm in the pen the loins with $pH_1 < 6.2$ showed a reduced frequency of about 33%. Calmness was, however, recorded only when the pigs were less than 1 hour in the pens. If, however, heavy turmoil occurred, the frequency of low pH_1 was above average irrespectively of the length of detainment.

In order to learn more about the effect of turmoil on the pigs we looked into the possible relationship between the appearance of the carcass with respect to bruises and scratches and the pH_1 of the loin. The carcasses under study were of pigs delivered from the progeny testing stations "Sjaelland" and "Jylland". Bruises mainly arise from pushes or blows delivered the animals by men, and perhaps fellow pigs, while driving the pigs from lorry to pen or from pen to sticking floor. All bruises of a size bigger than 5 cm^2 are recorded. Scratches mainly arise from the teeth when the pigs attempt to bite each other — sometimes also from the hoofs when a pig attempts to climb on another pig.

In Figure 6 increase or decrease of loins with pH_1 below 6.2 is given in percentage of the average frequency of the station. In the columns are given the number of observations. One notices that the frequency is increased for the pigs with bruises especially when bruises and scratches are combined. When the carcasses have no bruises or scratches the frequency is reduced. When the carcasses have only scratches the frequency is not substantially affected, for "Jylland" even decreased compared to the average frequency. The effects of the impacts on the animals giving rise to bruises are especially notable for "Jylland". This is interesting in view of the fact that the average level of frequency was somewhat lower for "Jylland" than for "Sjaelland". A number of factors probably contribute to increasing the frequency. When the impact of other factors are slight, the effect of the factors under study will be more spectacular.

These results suggest that the processes in the live pig staging the rapid fall in pH in the loin are to a considerable extent governed by fright and shock, rather than the mere exercise, of turmoil and driving.

When the pigs are excited as a consequence of turmoil, they will be correspondingly difficult to drive and bruises may result.

These observations are somewhat in line with observations made by Howard and Lawrie *(17)* on steers. "Excitability" rather than exercise brought about glycolysis in the muscles. For the steers the glycolysis resulted in depleted glycogen reserves while for the pigs under our study the glycolysis manifested itself in the rapid pH fall post mortem.

There seems to be a slight tendency towards an increased frequency of low pH_1 in the loins among the sows as compared to the barrows (Fig. 7). The increased frequency is probably due to some of the sows being in heat when delivered. During the heat period hormones, especially progesterone, are released from the ovaries and increase the metabolism of the animal. Wade *(29)* reports that liver mitochondrial ATPase was activated by progesterone at concentrations as low as 10^{-5} M. Other steroid hormones did not activate. As far as seasonal variations are concerned the frequency of

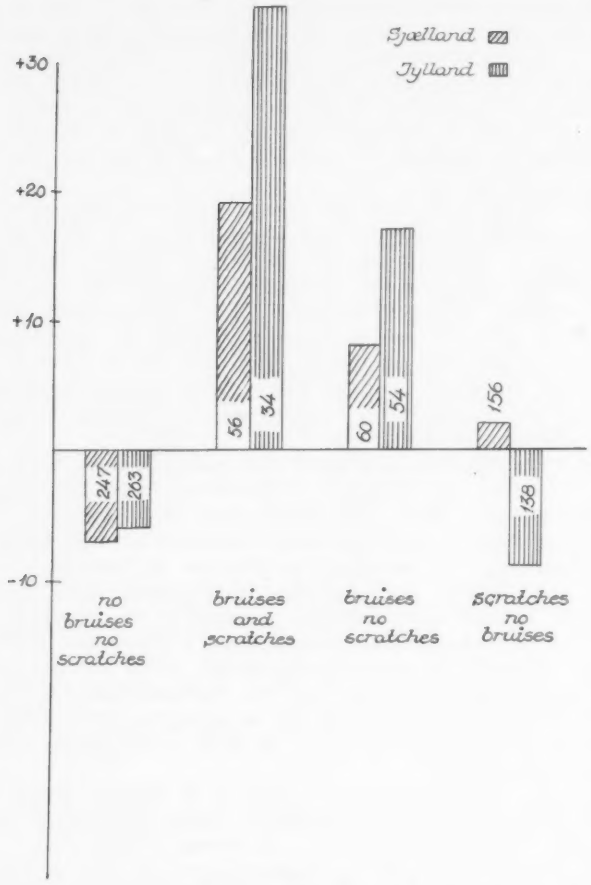

Figure 6. Percentage increase or decrease of loins with $pH_1 < 6.2$.

pigs giving loins with pH_1 below 6.2 are increased in the summer time, especially in hot months.

In Figure 8 variations in the frequency of pigs with pH_1 below 6.2 in the loin are recorded for winter, spring, and summer 1958 around the average frequency for the whole period. Under study were 830 pigs from "Sjaelland".

The same variation in frequency has been found in cases of real "muskel-degeneration" and has been attributed to decreased activity of the thyroid gland *(23)*. An effect to the thyroid hormones may be due to their effect on the oxygen consumption of the animal *(10)* or their effect on the ATPase activity of the myosin *(11)*. The increase in frequency may, however, also be due to increased excitability among the pigs when the weather is warm. The percentages of pigs with bruises for the 3 seasons as indication of the degree

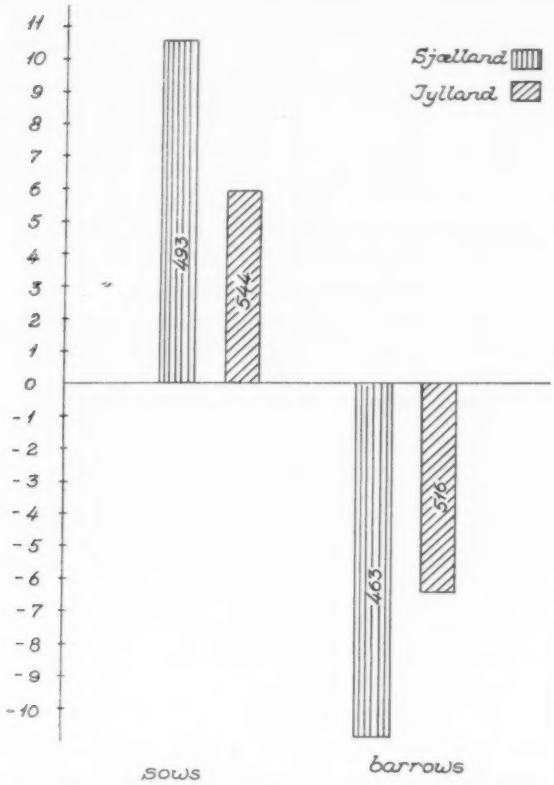

Figure 7. Percentage increase or decrease of loins with $pH_1 > 6.2$.

of "excitability" are given in Figure 8. It appears that the percentages are higher in the warm season.

EFFECT OF THE DIFFERENCE IN STRUCTURE ON THE QUALITY OF FRESH MEAT

To what extent does the consumer perceive differences in the quality of pork with different water holding capacities? To gather information on this point the colour was appraised in the fresh cut of the loin at the last rib the day after slaughter. The taste and texture was evaluated in fried chops cut from the back part of the loin.

Analytical methods

The meat colour. The appraisal was done by placing the meat in one of 7 colour classes to which there was assigned colour scores from 0.5 to 5 *(9)* . The classes were:

	Colour score	Description
1	0.5	Grey, the same colour as boiled meat, Very wet appearance.
2	1.0	Very pale pinkish, wet appearance
3	1.5	Pale pink, slightly wet appearance
4	2.0	Slightly paler than desirable
5	2.5-3.0	Desirable red colour
6	3.5-4.0	Slightly darker than desirable
7	4.5-5.0	Very dark

The haematin content of the meat was determined according to Lawrie *(21)*. As standard an alkaline solution of haemine (Hoffmann-La Roche) was used.

RESULTS AND DISCUSSION

Colour. The impression one gets of meat colour will partly depend on the pigment concentration, partly on the meat structure. Figure 9 shows the

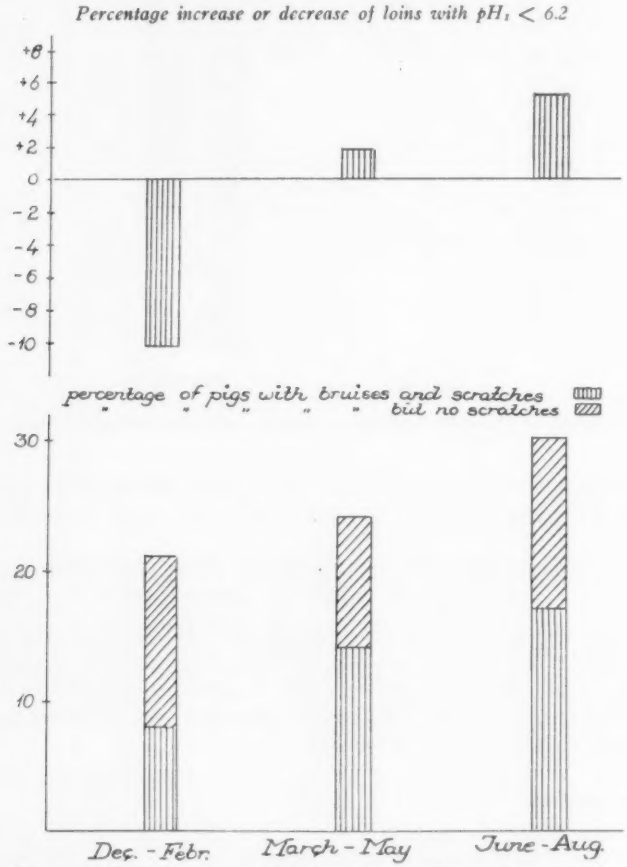

Figure 8. Variations as recorded for winter, spring, summer (1958).

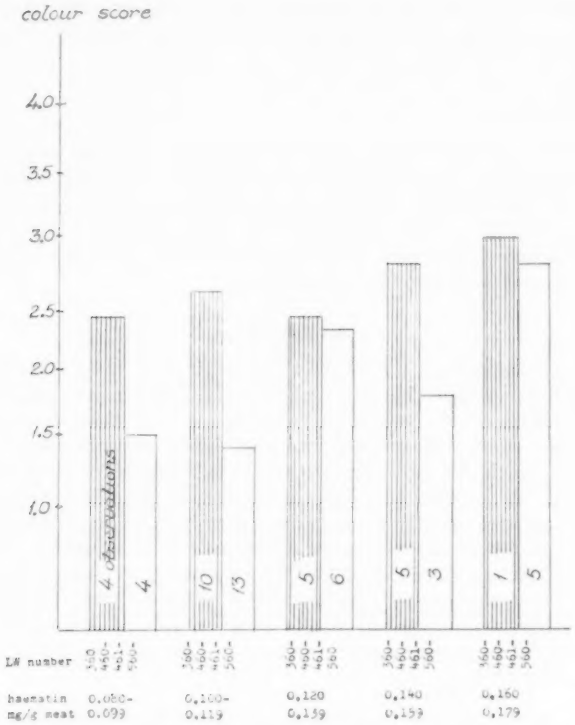

Figure 9. Average colour of the loin from a number of pigs.

average colour of the loin from a number of pigs, in accordance with meat structure, denoted by the LW number, and the haematin concentration which is taken as a measure of the pigment concentration. The examined loins were first grouped after haematin content; then each group was split in two sub-groups of loins with LW number below and above 460, respectively. For each subgroup the average colour score was calculated and illustrated with the length of the column. All loins examined had colour scores between 1 and 3. One notices that the meat with LW numbers below 460 have obtained colour scores about 2.5-3.0 irrespectively of the pigment concentration. The average score rose from 2.5 to 3.0 with increasing pigment concentration. For the meat with LW numbers above 460 the average colour score for the two groups with lowest haematin concentration was about 1.5, i.e. the meat had a pale colour. In the groups with higher pigment concentrations the colour was on an average better, but the results showed considerable variations.

In general one may conclude that the meat with normal water holding capacity has desirable colour even at the lower pigment concentration. When the structure is changed as illustrated in LW numbers above 460 the meat

has pale colour for the most usual pigment concentrations. With higher pigment concentrations the meat may, however, have desirable colour in spite of the adverse structure.

Taste and texture

A trained taste panel of 8 members examined 23 fried pork chops cut from loins with LW values in the interval 400-550. The results indicated that the meat was absolutely acceptable for consumption over the entire interval. There was, however, a slight decrease in the scores for taste as well as texture as the LW numbers increased. There appeared to be no leap in these quality characteristics at LW numbers around 460.

SUMMARY

Some observations on the meat quality in the loin from Danish landrace pigs have been made. The pigs under study were reared and fed under uniform conditions. For a part of these pigs, a very rapid fall in pH post mortem was recorded. In an hour the total fall in pH had almost occurred. The meat in which this rapid pH drop occurred had decreased water holding capacity and the proteins had lost a part of their solubility in 0.6 M KCl. The rapid fall was caused by accelerated glycolysis. The frequency of pigs showing rapid fall in pH post mortem appeared to be related to the amount of stress the animals were exposed to before slaughter. Excitability appeared more important than mere excercise. The effect of the decreased water holding capacity on the general quality of the meat was evaluated. The raw meat colour was in general paler whereas taste and texture of the fried chops were not essentially affected.

Acknowledgments

Various members of the staff of the Danish Meat Research Institute have assisted in the studies described in this paper. The author is particularly indebted to Mrs. Rosenbom for laboratory assistance and to Mr. T. Wichmann Jorgensen and Mr. O. Aalund for technical assistance. The author is grateful to The Research Laboratory on Animal Husbandry and the Roskilde bacon factory for kind collaboration throughout the studies.

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RELATIONSHIP OF MEAT PIGMENTS TO LIPID OXIDATION [a] [b]

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(Manuscript received May 9, 1958)

Oxidation of tissue lipids contributes markedly to undesirable flavor changes that occur in stored cooked meat products. Thiobarbituric acid values determined directly on the meat tissue increase rapidly in uncured cooked meats within a few hours after cooking and this increase is accompanied by development of off odors and flavors *(8)*. This oxidative reaction will be designated as "tissue rancidity" since the available evidence indicates that the site of oxidation is a protein bound phospholipid fraction not readily extractable with fat solvents.

This oxidative reaction is greater in uncured than in cured meats. Observations on cured meats in this laboratory suggested that they may be held at refrigerator temperature for a much longer period of time before tissue rancidity is noted. It was considered possible that differences in the heme pigments of cured versus uncured cooked meats might be responsible for this observed difference in their oxidative behaviour.

The catalytic effect of hemoglobin and other iron porphyrins on the oxidation of lipids is a generally acepted phenomenon. Earlier work, reviewed by Watts *(13)*, demonstrates that this reaction brings about destruction of the pigment as well as oxidation of the fat.

The mechanism of the reaction is incompletely understood. Banks *(1)* suggested that the active catalyst results from a combination of fat peroxide and iron porphyrin. In the most recent of a series of contributions by Tappel and coworkers, Maier and Tappel *(5)* propose the theory that catalytically active hemes form unstable compounds with fat peroxides, which then decompose to give two free radicals, each of which is capable of initiating an oxidation chain.

There is no clear evidence in the literature of the role of the cured meat pigment, nitric oxide hemochromogen, in the oxidation of unsaturated fats. Chang and Watts *(3)* showed that the addition of nitric oxide hemoglobin to model systems accelerated rancidity. Since their pigment preparation was very unstable, however, it is not clear whether nitric oxide hemoglobin or its ferric oxidation product was responsible for the observed catalysis. Tappel *(7)* reports similar catalytic effects of extracts from cured pork, but here again the pigment actually responsible for the catalysis is not known. The denatured cured meat pigment is not soluble in water.

It may be hypothesized that the cured meat pigment, in which the 5th and 6th coordination places of the iron are occupied by denatured globin and nitric oxide, respectively, would not be expected to react with a fat

[a] Data taken in part from dissertation submitted by Margaret J. Tims to the Graduate School of Florida State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[b] Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, Illinois, May 27, 1958.

peroxide in the manner postulated for hematin or hemoglobin. Evidence for this hypothesis is difficult to obtain with purified systems because of the insolubility of the pigment.

The work reported herein was designed to study lipid oxidation and accompanying odor changes in cured versus uncured cooked pork. It was hoped that the results might throw light on the catalytic activities of the heme pigments in meat and have a more direct applicaion to problems of meat storage than does work on solutions of heme pigments.

METHODS AND MATERIALS

Preparation of hams. Fresh pork hams were purchased from a local slaughtering house. Previous histories were unknown. The meat was ground once, using the attachment for the Kitchen-Aid mixer, Model 3-C. After grinding, it was thoroughly mixed by hand in order to obtain a homogeneous product. Curing salts, when used, were in the proportion of 2.0% sodium chloride and 0.02% nitrite ion in the form of sodium nitrite. A phosphate antioxidant was added to some samples. Sodium tripolyphosphate, $Na_5P_3O_{10}$, at a concentration of 0.5% *(14)* was the salt used. All salts were thoroughly blended and added to the raw meat in the dry state. Weighed portions of meat (500 g.) were packed tightly into No. 2 C-enameled cans, sealed immediately, and cooked in a steam bath to an internal temperature of 70° C. *(8)*. The time required for cooking was approximately one hour. After cooking, the cans were opened immediately, reground, thoroughly mixed, and stored at refrigerator temperature for subsequent assays.

Assay techniques. Oxidative rancidity was measured by the 2-thiobarbituric acid (TBA) test as outlined by Turner *et al. (9)* with a slight modification in that optical densities were read on the Evelyn photoelectric colorimeter using a 540 filter. The procedure for studying fat oxidation in artificial aqueous systems has been described elsewhere *(6, 11)*.

A panel of judges rated the intensity of rancid odor on the following scale: *not detectable, just detectable, moderate, moderately strong, strong,* and *very strong (2)*. A freshly cooked sample of meat containing a phosphate antioxidant served as a reference standard at each test period. This meat was from the same mixed lot as the experimental sample but had been frozen. A sample was removed from the freezer for each test period. Judges compared the odor of the test sample with that of the standard. An unidentified duplicate of the reference was included among the experimental samples. A judge's ability to identify the reference served as the basis for selection of the panel. Numerical scores of 6 to 1 (6, *not detectable; 1, very strong)* were assigned to the ratings given by the judges and averages of all judges were calculated.

EXPERIMENTAL RESULTS

Correlation of TBA values and organoleptic scores of cooked pork. In earlier work *(8)* in which a rapid oxidative reaction was observed in cooked pork, off odors were noted but no organized organoleptic data were obtained. Before comparing oxidative rancidity in cured and uncured meats, it was considered desirable to determine the correlation between TBA values and off odors in cooked meats by means of a sensory panel. Such data should be valuable in indicating the TBA value at which rancid odors are detectable.

Samples of pork which had been cooked to 70° C. and 110° C. with and without phosphate antioxidant were submitted to a panel of judges over a period of 10 days. Intensity of the rancid odor was evaluated. TBA values were determined on the samples each time they were submitted to the judges.

The average organoleptic scores and TBA values for all samples, regardless of treatment, are plotted in Figure 1. The sensory scores for samples having a TBA value below .40 were averaged and this value was used as the starting point of the curve. This group included 12 samples with average sensory scores ranging from 4.6 to 6.0 and TBA values ranging from .30 to .39. Many of these samples were antioxidant treated and retained the odor of freshly cooked meat throughout the test period.

A high degree of consistency was observed in the sequential ratings of the judges

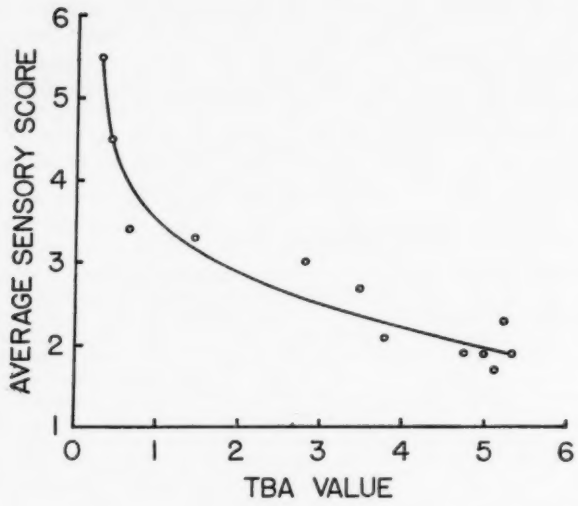

Figure 1. Relationship between TBA values and sensory scores in cooked pork.

although some variation was observed in the individual interpretation of the hedonic scale provided. The relative ratings of several samples by each judge were in line with the TBA values. Samples having average sensory scores of 4.5 to 6.0 (in the moderate to not detectable range of intensity of rancid odor) had TBA values between .30 and .40. When values reached the neighborhood of .45 to .60 or above, there was a sharp drop in acceptability ratings of the panel. This range appears to be the TBA value at which tissue rancidity is detectable. Turner *et al. (9)* observed that pork patties made with ground raw pork having a TBA value of .46 were judged to be of "borderline" quality while those with a TBA value over 1.20 were found unacceptable by the taste panel.

Fat oxidation in cured and uncured meat. The following experiment was designed to compare the development of tissue rancidity in cured and uncured meat. Portions of the same mixed lot of ground pork were cooked with (1) no addition, (2) a tripolyphosphate antioxidant, (3) curing salts and (4) curing salts plus tripolyphosphate. The results, reported in Figure 2, show that even in the absence of the antioxidant, oxidative rancidity did not occur in the cured meat during the two-week storage period. Polyphosphate inhibited oxidative rancidity in the uncured sample.

The possibility was considered that nitrite may interfere with the TBA reaction and that differences observed in the cured and uncured meat were due to an effect of nitrite on the test itself rather than on inhibition of fat oxidation. To test this, various concentrations of sodium nitrite were added to a sample of uncured cooked meat which had been stored at refrigerator temperature for several days. The TBA test was performed immediately after the addition of the nitrite. A concentration of 0.02% nitrite inhibited the reaction slightly giving a TBA value of 3.77 as compared to a control of 5.38. Lower concentrations had no appreciable effect, those of .01%, .005% and .001% having TBA values of 5.34, 5.09, and 5.53 respectively. Hougham and Watts *(4)* have shown that approximately 30% of the nitrite added to pork hams prepared as described above is lost during cooking and that further losses occur on storing. It therefore seems unlikely that the low TBA values obtained on cured meat can be attributed to an interfering effect of nitrite on the reaction.

Further evidence that the addition of curing salts resulted in protection of tissue lipids was obtained by organoleptic comparisons of cured and uncured meat. Samples of ground pork which had been cooked with (1) no addition, (2) a tripolyphosphate antioxidant, and (3) curing salts were submitted to a panel of judges for organoleptic

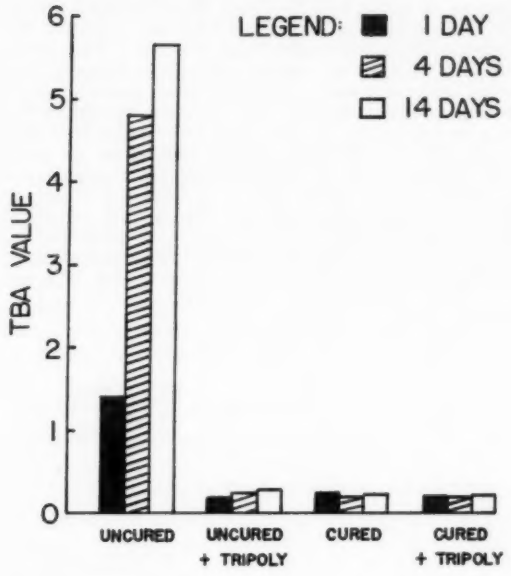

Figure 2. Lipid oxidation in cured and uncured refrigerated cooked pork.

ratings. TBA values were determined simultaneously. These data are recorded in Table 1. The sample containing curing salts consistently received high sensory ratings

TABLE 1
TBA values and sensory scores of cured and antioxidant treated samples of cooked refrigerated pork

Sample	Storage Interval					
	Initial		3 days		7 days	
	TBA value	Sensory Score [1]	TBA value	Sensory Score	TBA value	Sensory Score
1. Control	.49	3.3	3.49	2.2	2.82	2.6
2. Cured	.34	5.3	.34	4.9	.32	5.5
3. Antioxidant treated	.32	5.3	.32	4.7	.32	5.1

[1] Differences between 1 and 2, and between 1 and 3 are significant at the 1% level at all storage periods.

as did the sample in which the oxidative reaction had been controlled by the use of an antioxidant. TBA values for the 2 samples were comparable, and did not increase throughout the 7-day storage period. Off odors of the control sample were in the moderate to moderately strong range and were accompanied by high TBA values.

Since some of the nitrite in cured meat may be present in an aqueous phase in contact with animal fat, it might be argued that differences in rancidity in cured versus uncured meats are due to a direct antioxidant effect of the nitrite itself rather than to a difference in the heme pigments. This possibility was investigated in a model system in the absence of heme pigments. An artificial aqueous system was prepared in which

various concentrations of nitrite were incubated with fat containing carotene and rate of oxidation was followed by visual inspection of the fading *(6, 11)*. The time required for samples containing the highest concentration of nitrite (.02%) to turn rancid was 3 days as compared to 2 days for the control. This slight difference cannot account for the large differences observed in tissue rancidity in cured versus uncured meat.

Pigment fading and fat oxidation. The preceding experiments demonstate that oxidative reactions occur less rapidly in cured meats which retain their pink color. It might be expected that oxidation of the cured meat pigment to the ferric form would result in catalysis of fat oxidation. In the following experiment sodium chloride and nitrite were included separately and in combination in a sample of ground pork in an effort to vary stability of the cured meat pigment. The beneficial effect of salt on color stability of cured meats has been observed under some conditions *(10)*, although no adequate explanation for its effect has been offered. Visual observations of color changes and rate of fat oxidation (TBA) were followed. See Figure 3. The cured meat pigment

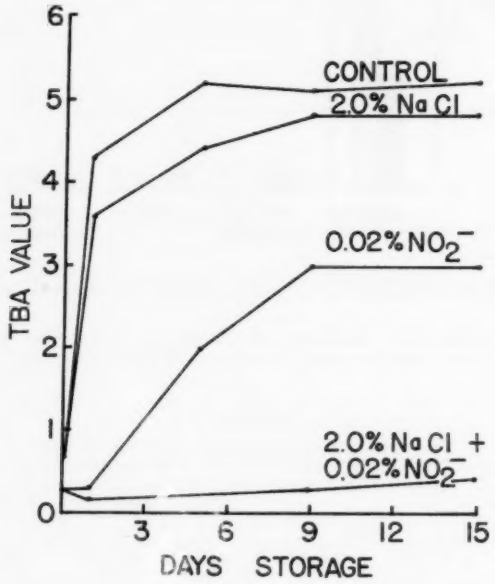

Figure 3. Effect of curing ingredients on TBA values of cooked pork.

developed in the samples containing nitrite as well as in those containing a combination of sodium chloride and nitrite. It was more stable when both salts were added and faded only slightly throughout the 15 day storage period. The sample which contained only nitrite faded rapidly after 24 hours and had developed a brownish gray color in 5 days. An increase in TBA value paralleled loss of color.

Discussion

The results of these experiments may be explained by the hypothesis that ferric hemochromogen is an active catalyst for unsaturated fat oxidation, whereas ferrous nitric oxide hemochromogen is not. During the cooking process of fresh meat all pigment is converted rapidly to the active catalyst,

grayish brown trivalent iron hemochromogen. In cured meat the concentration of the active catalyst is never so high. Just after curing all pigment is present in the ferrous form. Loss of the cured meat pigment may result from (1) oxidation to the active ferric form or (2) prophyrin ring destruction (loss of catalyst). Fading of color is a gradual process and both reactions may proceed simultaneously.

In the experiments reported above, the color of the meat cooked with both sodium chloride and nitrite remained pink until the beginning of bacterial spoilage. However, stability of cured meats to oxidative changes varies greatly. In previously reported work *(12)* involving 16 cured hams treated in various ways, the time required to reach a TBA value of 0.5 ranged from less than 2 days to greater than 14 days. In each case, however, loss of pink color accompanied fat oxidation. TBA values greater than 1.5 were rarely observed in cured meats although values ranging from 4.0 to 6.0 are commonly noted in uncured cooked pork.

Summary

The relationship of cured and uncured cooked meat pigments to oxidation of tissue lipids has been studied. Evidence is presented which suggests that the ferric form of the pigment is the active catalyst in tissue rancidity. Uncured cooked meat, which contains ferric denatured globin hemochromogen, showed high TBA values shortly after cooking. No such increase was noted in cured meat samples in which the pigment was present as pink ferrous nitric oxide hemochromogen. When brown ferric oxidation products appeared upon subsequent storage of the cured meats, TBA values increased. Rancid odors which develop in stored cooked meats were found to correlate with an increase in TBA values.

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EXTRACTION RATES FOR SELECTED COMPONENTS IN COFFEE BREW[a]

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Knowledge of extraction rates for the major components of coffee in contact with water and the effect of time and temperature thereon is important to the coffee industry inasmuch as it would provide a more objective measure of cup quality which in turn would aid in the improvement of coffee as a beverage.

Little basic information on extraction rates exists in the literature. Prescott *(12)* found that the best temperature range for a high quality coffee beverage lay between 185 — 203° F. (85 — 95° C.) and that the contact time between grounds and water should be relatively short. Under these conditions the caffeine was almost completely extracted and bitterness induced by temperatures at or near boiling and prolonged extraction was absent. Lockhart *(5)* noted that the rate at which each chemical component in coffee in contact with water is dissolved "is not only different but is directly dependent upon the temperature of the extraction." Natarajan, Nair, Rao, and Bhatia *(11)* found that extraction of chlorogenic acid varied with temperature; the higher the temperature the greater was the extraction of chlorogenic acid into the brew. Their observations were for a contact time of five minutes at temperatures of 209° F. (98° C.), 185° F. (85° C), and 167° F. (75° C).

This report describes an investiagtion of extraction rates for selected components in coffee brew; namely, soluble solids, color, caffeine, trigonelline, chlorogenic acid, and tannin for time intervals of 0.5, 1, 2, 5, and 10 minutes, and for temperatures of 200° F. (93° C.), 180° F. (82° C.), 160° F. (71° C.), 140° F. (60° C.), 120° F. (49° C.), and 100° F. (38° C.).

EXPERIMENTAL

Material. The coffee for this study was a "fine grind" *(6)* commercial blend received from The Coffee Brewing Institute, Inc., vacuum-packed in one-pound cans. It was stored at a temperature of −20° F. (−29° C.). Samples were removed from the storage room and equilibrated for 24 hours at 36-40° F. (2-4° C.) before use.

Preparation of brew. Brews were prepared with redistilled water in accordance with the formula recommendations of The Coffee Brewing Institute, Inc. *(13)*. The water was heated to boiling in a 1500 ml. Pyrex beaker covered with aluminum foil, and then cooled to a temperature slightly higher than that desired for a given brew, in order to correct for the cooling effect of the coffee itself.

The following chart gives the necessary information, found by experimentation, for obtaining a given brew temperature:

[a] Contribution No. 363 from the Department of Food Technology.

[b] Deceased September 24, 1959.

Brew temp. desired in °C.	Addition of coffee to water lowers temp. by number of degrees C. cited below	Temp. to which water should be cooled for desired temp.
93	4	97
82	4	86
71	3	74
60	2	62
49	1	50
38	1	39

For preparation of the 0.5 minute brew, the coffee was added and the brew stirred for 25 seconds. Exactly at the end of the 0.5 minute interval the brew was filtered from the grounds into a porcelain casserole of 1800 ml. capacity, covered with a single thickness of gauze. The one minute brew was prepared in exactly the same manner, but with an additional stirring for 25 seconds prior to collection time. The two minute brew was stirred initially, for 25 seconds prior to the one minute collection, and again for 25 seconds prior to the two minute collection. The same pattern was followed for the five minute and 10 minute brews. Fluctuation of temperature for a given brew was not greater than $\pm 0.5°$ C. The brews were filtered through Reeve Angel No. 812 folded filter paper. The first 25 to 50 ml. of filtrate were discarded and the remainder cooled to 68° F. (20° C.).

Methods of analysis. Methods referred to in this report were designed for the determination of various components in roasted coffee grounds. For the purposes of this study, these methods were adapted for the determination of brew components as well.

Soluble solids were estimated by the method of Lockhart *(5)*, using 15 ml. aliquots of the filtered brew. Total soluble solids were estimated in the coffee grounds by the method of Lockhart *(5)*, combined with an adaptation of the official A.O.A.C. method *(1)* to include 5 extractions with hot water and a contact time of two minutes for each extraction.

Color measurements were made with the use of a Beckman Model DU Quartz Spectrophotometer at a wave length of 405 mμ and a slit opening of 0.055 mm. The filtered brew was diluted 1:10 for reading. Total color was determined for the grounds by measuring the color of the filtrates of the corresponding soluble solids samples. The No. 1 extracts were diluted 1:5 for reading; the other extracts were read without dilution. Color was calculated by the following equation:

$$\frac{\text{optical density at } 405\text{m}\mu \times \text{dilution}}{\text{original wt. of sample}} = \text{color index per gram}$$

Caffeine was estimated by the spectrophotometric method of Ishler, Finucane, and Borker *(2)*. For analysis of the coffee brew, 20 ml. samples were used.

Trigonelline was estimated by the method of Moores and Greninger *(9)* which provided for chromatographic separation of the trigonelline and spectrophotometric measurement of the purified sample. For analysis of coffee brew, 25 ml. of filtered brew was diluted to a volume of 250 ml. with 50% ethyl alcohol and 100 ml. aliquots taken for adsorption on the Celite-Filtrol columns as in the case of coffee grounds. The method of extraction of trigonelline from the grounds was altered slightly to include percolation of 100 ml. of hot water through the adsorption tubes containing mixtures of grounds and Celite, followed by 100 ml. of 50% ethyl alcohol as in the regular method. This change was made after it was found that trigonelline values in coffee grounds were lower than those obtained for 5 minute and 10 minute brews extracted at 200° F. and 180° F., which indicated that for trigonelline determination on the grounds some preliminary extraction with heat was necessary.

Chlorogenic acid was estimated by the method of Weiss *(15)*. For analysis of coffee brew, 20 ml. of filtered brew was diluted to 500 ml. to provide solution A of the method. This solution was further diluted 1:10 for spectrophotometric measurement. The method of Moores, McDermott, and Wood *(10)* is essentially the same but employs room temperature extraction and was originally used until it was found that chlorogenic acid values for 10 minute brews extracted at 200° F and 180° F were higher than those obtained in the coffee grounds. When the hot extraction method of Weiss *(15)* was employed, consistent results were obtained.

Tannin was estimated by the official A.O.A.C. method for tannins in tea and coffee *(1)*. For analysis of coffee brew 150 ml. of filtered brew was diluted to 500 ml. and 10 ml. aliquots taken for titration, as in the case of coffee grounds. For the 0.5, 1, and 2 minute brews, prepared at the 4 lower temperatures, 200 ml. of filtered brew was diluted to 500 ml. to increase the sensitivity of the method.

Moisture in the coffee grounds was estimated by the method of Schwartzman *(14)*.

Sampling of the coffee grounds was made without further sieving to provide data comparable with those obtained in the brew prepared from the grounds. Each component analysis was carried out in triplicate. All data were calculated to a dry weight basis. Extraction rates for the selected components in coffee brew were calculated in terms of per cent extractable, using the data obtained from the coffee grounds as the reference base.

RESULTS AND DISCUSSION

Data obtained for the selected components of coffee brew are presented in Table 1. The data obtained for the roasted coffee grounds are presented in Table 2.

The values for caffeine and trigonelline in coffee grounds (Table 2) are in agreement with values for coffee blends reported by Ishler, Finucane, and Borker *(2)*, Moores and Greninger *(9)*, and Segall and Proctor *(13)*. The values for these components are also in agreement with those reported by Kogan, DiCarlo, and Maynard *(4)* who combined paper chromatography with spectrophotometry in their analyses.

The values for chlorogenic acid in coffee grounds (Table 2) are in agreement with those of Weiss and Steers *(16)* while the tannin values are in the same general range as those reported by Iyengar, Natarajan, and Bhatia *(3)* for Indian coffees. These authors found, however, that tannin values obtained by the A.O.A.C. method *(1)* are low. According to them, more consistent results are obtained when lead acetate is used instead of gelatin in the precipitation procedure.

Extraction rates for the selected components in coffee brew have been plotted as per cent extractable versus time in minutes for each time interval and each temperature, and are presented for soluble solids, color, caffeine, trigonelline, chlorogenic acid, and tannin, in Figures 1, 2, 3, 4, 5, and 6, respectively. For each component, these figures indicate that the higher the temperature, the greater is the amount of the component extracted.

With respect to time soluble solids, color, and chlorogenic acid (Figures 1, 2, and 5) increased over the 10 minute period of study, the increase being less pronounced after the first 2 minutes. The effect was most marked for chlorogenic acid; the least for color.

Caffeine values (Figure 3) for the two highest temperatures showed a tendency to level off after the first 2 minutes; for temperatuers of 160° F., 140° F., and 120° F. a gradual increase was shown over the 10 minutes period; for the 100° F. temperature, values began to level off after the first 2 minutes.

Trigonelline values (Figure 4) showed a fairly rapid increase for the first 2 minutes, followed by a more gradual increase and then leveled off. Time had no apparent effect after the first 5 minutes for this component at temperatures of 180° F., 160° F., and 140° F. The very slight difference for 200° F. was not significant.

Tannin values (Figures 6) showed a tendency to level off after the first two minutes for temperatures of 200° F. and 180° F. Marked increases

TABLE 1

Average values for selected components in coffee brew

Time in min.	Soluble solids Dry basis	Color dry basis per g.	Caffeine dry basis	Trigonelline dry basis	Chlorogenic acid dry basis	Tannin dry basis
200° F. temperature	(%)		(%)	(%)	(%)	(%)
0.5	13.8	73	0.77	0.69	2.69	1.06
1.0	15.2	81	0.86	0.76	3.00	1.23
2.0	17.3	93	0.99	0.83	3.54	1.36
5.0	19.5	108	1.07	0.88	4.15	1.52
10.0	20.4	113	1.10	0.89	4.37	1.57
180° F. temperature						
0.5	13.5	68	0.75	0.66	2.58	0.91
1.0	14.7	75	0.79	0.72	2.82	1.12
2.0	16.5	85	0.92	0.76	3.33	1.27
5.0	18.9	98	1.02	0.87	3.92	1.49
10.0	19.6	107	1.06	0.88	4.16	1.51
160° F. temperature						
0.5	12.8	63	0.67	0.58	2.36	0.51
1.0	13.4	67	0.71	0.67	2.51	0.63
2.0	15.3	73	0.81	0.73	2.96	0.84
5.0	17.9	90	0.98	0.88	3.72	1.00
10.0	19.0	94	1.07	0.88	3.84	1.37
140° F. temperature						
0.5	12.3	54	0.59	0.52	2.23	0.45
1.0	12.6	59	0.68	0.65	2.30	0.50
2.0	13.7	64	0.72	0.68	2.67	0.70
5.0	16.9	82	0.92	0.85	3.39	0.89
10.0	18.6	89	1.02	0.88	3.73	1.23
120° F. temperature						
10.0	11.0	50	0.57	0.51	2.08	0.30
0.5	12.2	53	0.67	0.64	2.22	0.47
1.0	12.9	58	0.67	0.66	2.63	0.65
2.0	15.7	71	0.86	0.81	2.98	0.83
5.0	18.2	80	0.99	0.86	3.49	1.17
100° F. temperature						
0.5	9.04	40	0.48	0.40	1.58	0.23
1.0	9.43	42	0.50	0.48	1.69	0.29
2.0	11.6	49	0.58	0.62	2.26	0.47
5.0	14.3	62	0.77	0.77	2.68	0.47
10.0	15.8	65	0.82	0.79	2.98	0.77

TABLE 2

Average [1] values for selected components in roasted coffee grounds

Component analyzed	Average values in % dry basis			Grand average
	Can No. 1	Can No. 2	Can No. 3	
Soluble solids	24.17	24.22	24.31	24.23
Corresponding color	164.5	166.6	166.3	165.8 [2]
Caffeine	1.24	1.24	1.25	1.24
Trigonelline	1.02	1.03	1.02	1.02
Chlorogenic acid	5.14	5.14	5.12	5.13
Tannin	2.09	1.99	2.00	2.04

[1] Data represent averages of triplicate determinations.
[2] Data represent color index per gram of coffee (dry basis).

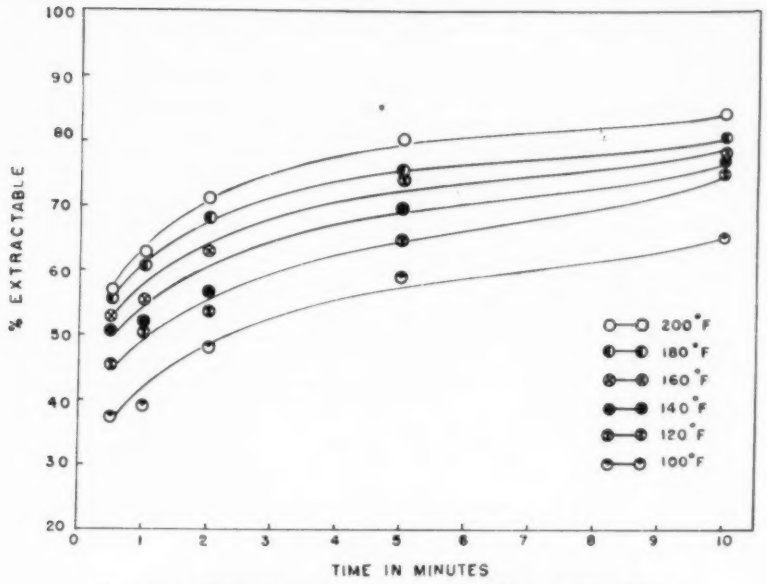

Figure 1. Extraction rates for soluble solids in coffee brew.

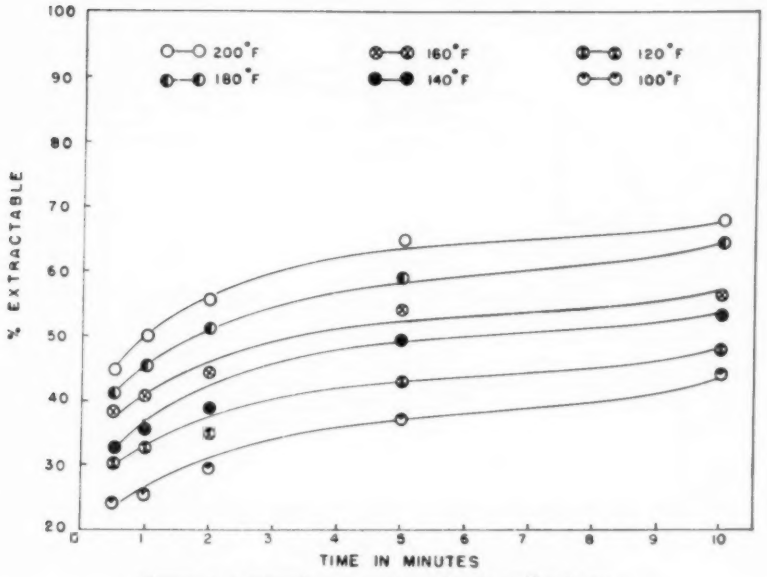

Figure 2. Extraction rates for color in coffee brew.

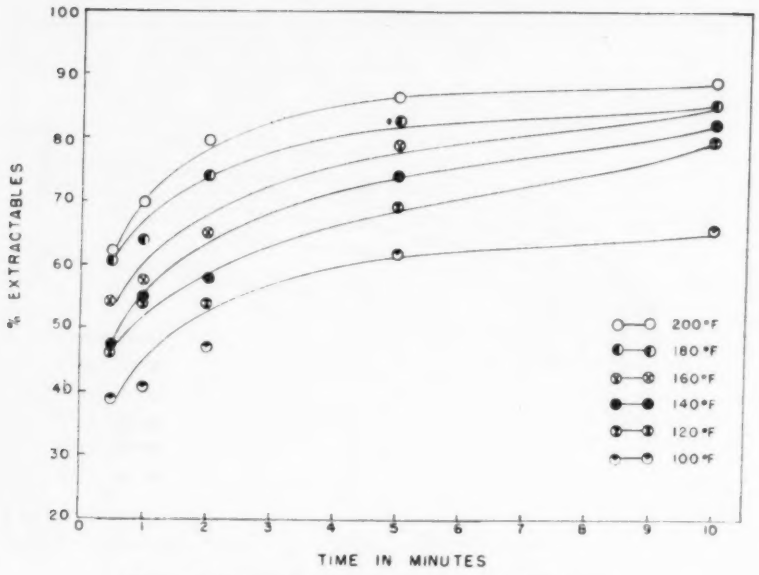

Figure 3. Extraction rates for caffeine in coffee brew.

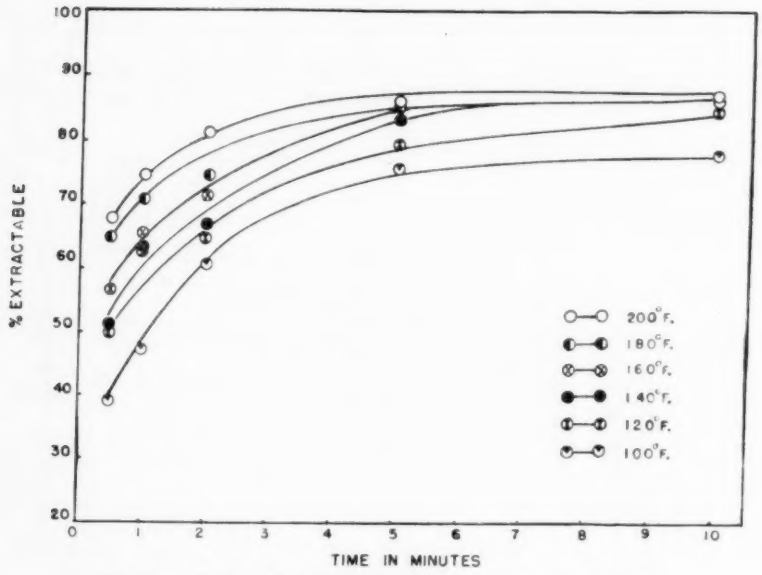

Figure 4. Extraction rates for trigonelline in coffee brew.

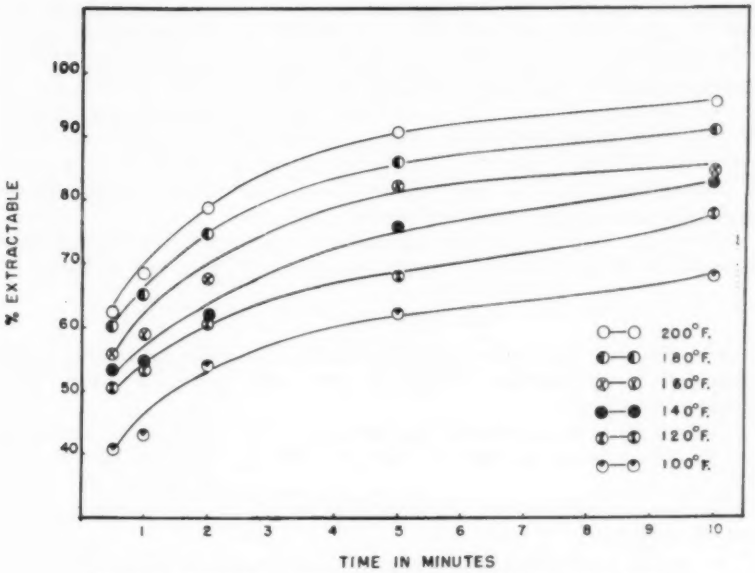

Figure 5. Extraction rates for chlorogenic acid in coffee brew.

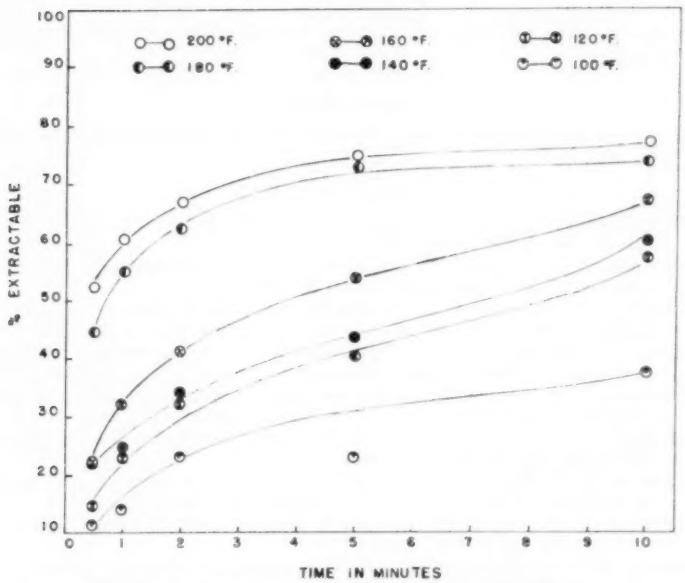

Figure 6. Extraction rates for tannin in coffee brew.

were shown, however, over the 10 minute period for temperatures of 160° F., 140° F., and 120° F., while the curve for 100° F. was comparatively flat.

A comparison of extraction rates for 2 minute contact times and 200° F. temperatures (the conditions normally met with in coffee brewing *(7)*), showed the highest rate for trigonelline (81.3%), followed by caffeine (79.8%), soluble solids (71.0%), chlorogenic acid (69.0%), tannin (66.8%), and color (56.0%). Therefore, it would appear that extraction proceeds at a more rapid rate for trigonelline and caffeine under normal brewing conditions than for the other components.

Natarajan *(11)* noted that caffeine was more readily extracted than chlorogenic acid in coffee brew which is in agreement with our findings.

SUMMARY

The effect of time and temperature on extraction rates for soluble solids, color, caffeine, trigonelline, chlorogenic acid, and tannin, in coffee brew, has been investigated.

Brew extracts were prepared for intervals of 0.5, 1, 2, 5, and 10 minutes, and for temperatures of 200° F., 180° F., 160° F., 140° F., 120° F., and 100° F.

Extraction rates for each component increased with increasing temperature of extraction.

In respect to time, extraction rates for soluble solids, color, and chlorogenic acid showed an increase over the 10 minute period of study, the increase being less pronounced after the first two minutes. Extraction rates of the components studied decreased in the following order: trigonelline, caffeine, soluble solids, chlorogenic acid, tannin and color. Time had no apparent effect on rate after the first five minutes for trigonelline at temperatures of 140° F to 200° F.

Acknowledgment

This study was made possible by a Grant-in-aid from the Coffee Brewing Institute, Inc., New York, N. Y.

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DISSOLVED OXYGEN DETERMINATION IN WINE

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Present methods of determining oxygen in wine have some drawbacks. The presence in wine of other easily reducible ions causes inaccuracies that are difficult or impossible to avoid with standard chemical *(1, 7, 8)* or polarographic *(5)* methods.

Hummel *(4)* reported the following gasometric method for beer. Oxygen dissolved in the beer is purged by raising the temperature and letting the evolved carbon dioxide sweep out the oxygen. The gases are then passed through a solution that traps the carbon dioxide, and the oxygen is measured by its displacement of water. Wine contains little or no carbon dioxide, but the same principle could be applied by sweeping with carbon dioxide. The main difficulties would occur in determining how long to sweep the wine and in measuring the amounts of nitrogen and other gases swept out and measured as oxygen.

The best chemical method available for determining dissolved oxygen in wines is one using indigo carmine indicator and reducing the dissolved oxygen under a blanket of nitrogen with hydrosulfite. If carefully used, this method is adequate and accurate *(2)* for measuring differences in relatively large amounts of dissolved oxygen in white wines, but it is very difficult to use with red or pink wines. The equipment and technique involved are somewhat elaborate for routine work, and the method measures combined as well as free oxygen.

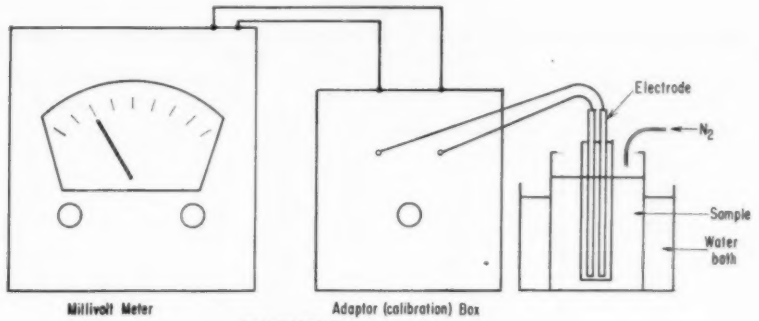
One winery laboratory currently uses a polarograph for oxygen determinations, although this method probably measures not only oxygen but also other ions reduced at the measuring potential. However, it is probably accurate in determining differences in the dissolved oxygen present before and after a wine transfer, which is the prime interest involved in this case.

A new method for dissolved-oxygen determination developed by Beckman Instruments Inc. *(9)*, based on the work of Clark *et al. (3)*, offers a promising solution to the problem.

The basic principle is a polarographic determination of oxygen after it has passed through a polyethylene membrane. This offers the advantage that other easily reducible ions in solution are screened from the electrodes.

METHOD AND MATERIALS

The measuring device used for this work was the Beckman Oxygen Electrode, Model 11098, with an adaptor assembly similar to their Model OM-2. The basic components of this electrode are a silver anode and platinum cathode, connected by potassium chloride solution. The two electrodes are screened from the outside by a polyethylene membrane that allows gases to pass through to the platinum cathode. The adaptor assembly is a voltage divider placing a potential of about 0.6 volt on the electrode. When all reducible materials have been used in the electrode, it becomes polarized. Any gas reducible at this potential coming through the membrane is reduced at the cathode, and current is transmitted to the anode and to some measuring device, such as a potentiometer or D. C. microammeter. Figure 1 shows a schematic diagram of the equipment setup.



EQUIPMENT

Figure 1. Schematic diagram of equipment.

Both Beckman Model G and Model H pH meters were used to read off the electrode. All readings were taken with the material in question in a water bath controlled to $\pm 0.1°$ C. Nitrogen was used to blanket all solutions that were less than saturated with air. Saturation or partial saturation of samples was accomplished by sparging with air. Deaeration of samples was either by sparging with nitrogen or by boiling and cooling under nitrogen.

Dilute sulfur dioxide samples were obtained by adding small amounts of near-saturated sulfur dioxide solutions to deaerated water.

Carbon-dioxide-saturated solutions were made by sparging with carbon dioxide gas.

The dissolved oxygen present in the deaerated and air-saturated water samples for the zero point and calibration point were determined by the Winkler method.

EXPERIMENTAL AND DISCUSSION

Effect of instrument and millivolt range. To find if the instruments gave linear readings, measured amounts of air-saturated water were added to a known amount of deaerated water under a blanket of nitrogen. The instrument was first set to zero and then set at a certain millivolt reading with air-saturated water at 25° C., and then the zero was rechecked and the calibration point rechecked. Figure 2 plots the results obtained for such measurements taken at 0-322, 0-200, and 0-150 millivolts on the Model H Beckman pH meter. It can be seen that, if the calibration point is brought down to 150 millivolts, the curve becomes linear. Similar tests on the Beckman Model G gave a non-linear curve at 0-350 millivolts, and a straight curve at 0-50 millivolts. No tests were made on other ranges with the Model G.

Table 1 shows a mean error of $+0.01$ cc. oxygen per liter and a standard deviation from the mean error of ± 0.07 cc. oxygen per liter for the values obtained on water calibration curves plotted at the 150 millivolt range.

Effects of wine types. Tests over the range of 0-150 millivolts were carried out with 3 different wines (dry red, dry white, and white sweet). Aerated and deaerated portions were mixed under nitrogen and the readings taken. The aerated sample was measured several times, and an average value was taken for calculation of the oxygen present in mixtures of aerated and deaerated samples. Figure 3 shows the calibration curve and the values found for the three wines.

The methods used for deaerating and aerating of the samples would have sparged out any possible interfering sulfur dioxide or carbon dioxide. The effects of solutions of these gases on the electrode are shown later in this paper. Nothing normally found in wine other than these 2 gases and hydrogen sulfide or oxygen would affect the electrode.

Table 2 summarizes the data obtained with the Model H over the range of 0-150 millivolts for the values plotted in Figure 3. These data show a standard deviation of ± 0.08 cc. oxygen per liter from the mean error of $+0.03$ cc. oxygen per liter for the 19 values tabulated.

Effect of sulfur dioxide and carbon dioxide. Investigation of the effects of sulfur dioxide on the electrode showed no effects in the range investigated (0-300 mg. per liter).

TABLE 1

Mean error and standard deviation from the mean error from calibration curves with water at 150 millivolt range

cc. of O_2 per liter				
Calculated amount	Determined amount	Difference (error)	Mean error	S.D.
1.91	1.92	+ 0.01		
2.05	2.07	+ 0.02		
2.55	2.60	+ 0.05		
2.87	2.84	− 0.03		
3.18	3.12	− 0.06		
3.43	3.45	+ 0.02		
3.45	3.45	0.00		
4.05	3.90	− 0.15		
4.32	4.34	+ 0.02		
4.45	4.45	0.00		
4.68	4.73	+ 0.05		
5.05	5.20	+ 0.15		
5.13	5.21	+ 0.08		
			+ 0.01	± 0.07

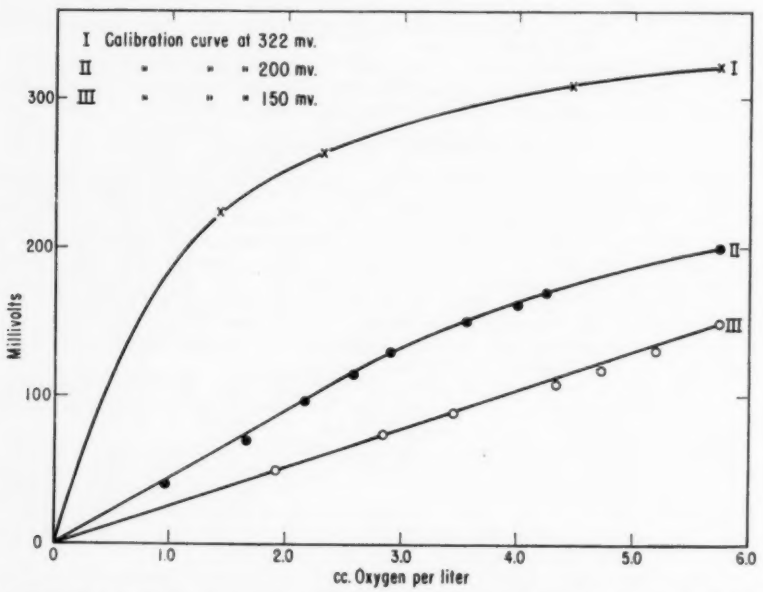


Figure 2. Calibration curves at various millivolt ranges with Beckman Model H pH meter.

Further investigation with a 3.6% solution of sulfur dioxide still showed no effect. The sulfur dioxide dissolved in water or wine *(6)* is almost completely in the bisulfite or acid form (HSO_3^-, H_2SO_3), with very little existing as SO_2 except at very high hydrogen-ion concentration. Similar tests with water saturated with carbon dioxide showed no effects on readings with the oxygen electrode.

TABLE 2

Mean error and standard deviation from the mean error

Wine type	Calculated amount (cc. of O_2 per liter)		Difference (error)	Mean error	S.D.
Dry red	1.37	1.62	+ 0.25		
	2.20	2.32	+ 0.12		
	2.75	2.90	+ 0.15		
	3.67	3.65	− 0.02		
	4.12	4.15	+ 0.03		
	4.40	4.35	− 0.05		
				+ 0.08	± 0.11
Dry white	1.13	1.10	− 0.03		
	1.70	1.76	+ 0.06		
	2.04	2.14	+ 0.10		
	2.55	2.55	0.00		
	2.79	2.84	+ 0.05		
	2.92	2.96	+ 0.04		
	3.04	3.10	+ 0.06		
				+ 0.04	± 0.04
White sweet	1.12	1.12	0.00		
	1.80	1.83	+ 0.03		
	2.25	2.22	− 0.03		
	3.00	2.94	− 0.06		
	3.38	3.33	− 0.05		
	3.60	3.54	− 0.06		
				− 0.03	± 0.04
Total				+ 0.03	± 0.08

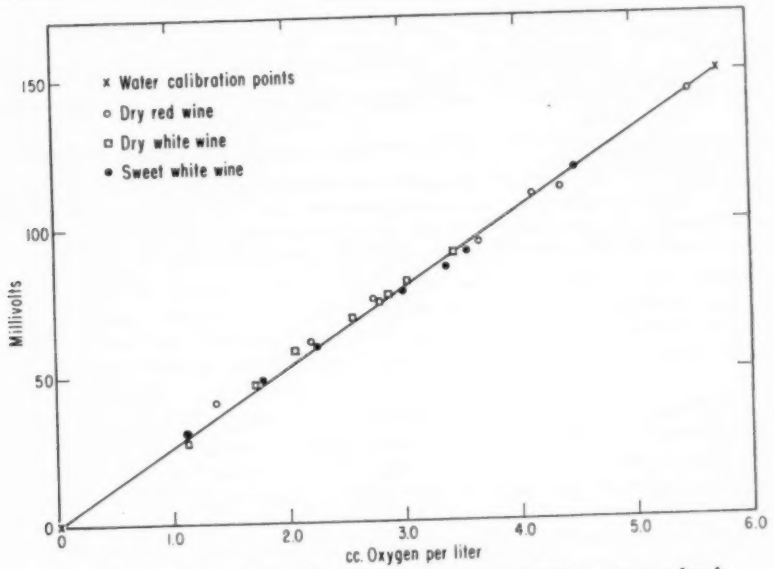


Figure 3. Values for three different types of wine at various oxygen levels.

Other factors. Watanobe and Leonard *(9)* said that current flow is directly proportional to membrane thickness. They also reported a temperature coefficient of 5% per degree Centigrade near room temperature.

The electrode is not very susceptible to general "poisoning" of the platinum. It can be used several days in either water or wine before cleaning is ncessary.

To obtain dependable readings, the electrode or the fluid should be in slight motion, thus assuring a maximum sampling surface for the membrane. Without such motion, readings will vary.

Response times will vary directly with the temperature, but at 25° C. the response time from 0 to 150 millivolts is about 90 seconds. Response time of 150 millivolts to zero is slightly longer. This longer response time is probably due to the time factor of the complete use of oxygen already passed through the membrane.

Hydrogen sulfide is reported *(9)* to poison the electrode, but the presence of this gas almost always precludes the presence of oxygen, so this phase was not investigated.

SUMMARY

It has been shown that, if proper precautions are taken, oxygen dissolved in wine can be determined with good accuracy and dependability. The presence of other dissolved gases generally present in wine (except hydrogen sulfide), such as sulfur dioxide or carbon dioxide, will have no effect on the readings.

By the method presented, straight-line calibration of the instrument results if the proper millivolt range is used with the pH meters.

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STUDIES ON THE RETENTION OF ASCORBIC ACID DURING BLANCHING OF SOME INDIAN VEGETABLES

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A considerable amount of ascorbic acid is lost during blanching of vegetables prior to canning. Some workers have reported different methods of blanching to minimise this loss *(1, 2, 4, 5, 7)*. In the present studies the effect of blanching of some common Indian vegetables in different media on the retention of ascorbic acid has been reported. The samples were purchased from the local markets. These were washed, dried by a towel and cut into pieces suitable for canning. Ascorbic acid of the raw samples were estimated; 100 g. portions of the samples were then blanched for 4 min. at 82° C. in a beaker in

1) 300 ml. of water,
2) 300 ml. of 0.11% sodium sulphite (on anhydrous basis) solution, the sulphite being added just before immersing the vegetables,
3) 300 ml. of water after dipping the vegetables in 4 vols. of 0.5% sodium sulphite solution for ½ minute at room temperature (26 — 30° C.),
4) 300 ml. of water after dipping the vegetables in 4 vols. of 0.075% sodium sulphite *plus* 5% brine solution for 1½ minutes at room temperature,
5) 300 ml. of lactic acid solution, pH 3.5.

After blanching the materials were strained, cooled and representative samples were taken to estimate the ascorbic acid. Ascorbic acid was estimated by titration against 2 : 6 dichlorophenol indophenol according to the method of Harris and Olliver *(3)*. When sulphite was present the method was modified as described by Mapson *(6)*.

Results on the percentage retention of ascorbic acid after blanching according to the methods given above are given in Table 1. The results are given on the average of 6 samples analysed.

It will be found from the result in Table I that blanching in an acid (lactic acid, pH 3.5) medium had the best effect on the retention of ascorbic acid. Effect of blanching in 0.11% sodium sulphite (anhydrous) solution was next best. Dipping in sodium sulphite solution with or without sodium chloride and then blanching in water had also a beneficial effect on the retention of ascorbic acid but less than blanching in acid medium or in sulphite solution.

It was observed during the experiments that the culinary qualities deteriorated when the samples were blanched in acid medium. Blanching in sulphite solution produced a slight improvement while dipping in sulphite solution with or without sodium chloride before blanching in water had little effect on the culinary qualities. It would thus appear that in order to combine the retention of ascorbic acid with culinary quality, the method of blanching in sodium sulphite solution under the stated conditions appears to be the best.

TABLE 1

Sl. No.	Names of vegetables [1]	Ascorbic acid content *mg./100 g.*	% retention of ascorbic acid after blanching by method				
			1	2	3	4	5
1	Patol *(Tricosanthes dioica Roxb)*	38.1	76.4	86.7	80.9	83.0	90.6
2	Cabbage *(Brassica oleraceacapitata)*	53.9	60.5	70.5	66.8	70.5	70.5
3	Bittergourd *(Momordica charantia)*	118.6	61.6	78.2	73.9	79.1	78.6
4	Kankrol *(Momordica cochinchinensis spreng)*	312.0	70.6	89.6	81.7	87.9	94.7
5	Barbati *(vigua cylindrica skeels)*	18.4	75.0	4.8	81.5	82.0	92.3
6	French Beans *(Phaseolus vulgaris)*	7.1	69.0	86.0	75.2	74.6	95.6
7	Cluster Beans *(Cyamopsis tetragonoloba)*	9.7	55.6	75.2	70.1	68.4	75.2
8	Potato *(Solanum tuberosum)*	25.3	76.3	86.5	83.0	83.9	89.7

[1] Patol, Kankrol, and Barbati are the local names in Bengali.

Acknowledgments

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EFFECT OF COOKING RICE WITH DIFFERENT VOLUMES OF WATER ON THE LOSS OF NUTRIENTS AND ON DIGESTIBILITY OF RICE *IN VITRO*.

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It is the usual practice in oriental countries to wash rice before cooking in order to remove dust and other foreign material. The washed rice is cooked in some areas with the minimum quantity of water which is entirely absorbed by the cooked rice, but in certain other areas with excess of water. The gruel [a] is drained after cooking. These operations — washing, cooking and draining the gruel — entail losses of nutrients. The present investigation was undertaken to study quantitatively the losses of thiamine, riboflavin, niacin, calcium, phosphorus, phytin phosphorus, iron, available iron and nitrogen during washing and cooking of some pure strains of rice with different volumes of water. Some work has been done from time to time on this subject *(1, 4, 11, 17)* but a systematic investigation on pure strains of rice with regard to almost all the nutrients does not appear to have been made.

MATERIALS AND METHODS

The strains which were rich in thiamine were selected for this experiment from the list published by Banerjee and Guha *(5)*.

The pure strains were obtained from the Agricultural Directorates of different State Governments as paddy. The strains were hulled in a P. S. G. Sheller [b] at a standard speed of 90 strokes per minute only to remove the husk and the rice was taken for analysis.

Rice (200 g.) was first washed in a beaker with 200 cc. of distilled water, the water drained and the washing repeated twice more. The wash-water was made to a definite volume and aliquots were taken for the analysis of thiamine, riboflavin, niacin, iron, available iron, protein, calcium, phosphorus, and phytin phosphorus. The washed rice was then cooked in an aluminum vessel with lid on with 6 volumes (1200 cc.), 8 volumes (1600 cc.) and with just sufficient volume of water (which varied with different samples). The lid was removed as soon as boiling began and the cooking continued until the rice became soft enough for consumption. When cooked with excess water the cooked rice was drained for a definite period of time (10 min.). The gruel was made up to a definite volume and the weight of the cooked rice recorded. Aliquots of gruel and of the cooked rice were taken for the estimation of thiamine, riboflavin, niacin, iron, nitrogen, calcium, phosphorus and phytin phosphorus.

For the estimation of thiamine the method of Harris and Wang *(8)* was followed. Riboflavin was estimated according to the method of Vitamin Chemists, Inc. *(13)* and the determination of nicotinic acid was made by the method of Chaudhuri *(6)*.

Nitrogen was estimated by the usual Kjeldahl method. Total phosphorus in the ash was estimated according to the method of Fiske and Subbarow *(7)* and that of phytin phosphorus according to the method of MaCance and Widdowson *(12)* using a 'Lumetron' photoelectric colorimeter. Calcium was estimated from the ash following the method described in the official methods of the A.O.A.C. *(14)*. Total iron was estimated following the method described by Hummel and Willard *(10)* after ashing the sample and available iron by the method of Hill *(9)* as modified by Saha and Guha *(16)*.

[a] That is, the water poured off after cooking.

[b] P.S.G. sheller — manufactured by P.S.G. Co., Ltd., Coimbatore (S.I.)

For the digestibility experiments quantities of cooked rice equivalent to 2.5 g. of uncooked rice were taken in a tissue homogeniser, ground well and made up to 50 cc. with water. 10 cc. aliquots of this suspension (equivalent to 0.5 g. of uncooked rice) were incubated separately in glass stoppered flasks with (a) takadiastase using acetate buffer of pH 4.6 and with (b) pancreatin using phosphate buffer of pH 6.9. The amount of reducing sugar was estimated before and 1 hour after adding the enzyme preparation by the method given in A.O.A.C. *(15)*. The reaction was stopped by adding 1 cc. of 2N sulphuric acid and the protein precipitated by adding 1 cc. of 12% sodium tungstate solution. The volume was made up to 20 cc. and the mixture filtered. The amount of reducing sugar produced per cc. of the filtrate was estimated. The cooked rice which produced more sugar was considered to be more digestible.

RESULTS

Thiamine, riboflavin, niacin, nitrogen, calcium, phosphorus, phytin phosphorus, iron and available iron contents of the rice samples investigated are given in Table 1.

The loss of nutrients in the washing of rice with water before cooking is given in Table 2.

Losses of nutrients in gruels during cooking of different strains of washed rice with 6 and 8 volumes of water in each case are given in Table 3. Retention of the nutrients in the cooked rice has also been estimated to check results of losses in cooking.

TABLE 1

Nutrient contents of rice samples

Samples	Charnock [1]	ADT3 [2]	T3 [3]	T26 [4]	Badsabhog [5]
Moisture %	10.8	10.6	12.9	14.1	9.6
Ash	1.48	1.5	1.45	1.11	1.25
Thiamine/μg/100 g.	480.48	460	600	530	500
Riboflavin/μg/100 g.	185	60	130	160	125
Niacin mg./100 g.	5.02	5.92	7.56	3.89	6.70
Calcium mg. %	20.0	17.0	21.0	18.0	20.0
Phosphorus mg. %	608.3	410.3	600	65.0	230.0
Iron mg. %	2.87	4.50	2.50	2.63	3.20
Available iron mg. %	2.75	2.75	2.00	1.60	1.88
Phytin phosphorus mg. %	460.0	280.0	372.0	35.0	150.0
Nitrogen %	1.90	1.75	1.60	1.63	1.64

1 *Charnock* — medium stature, fine white grains.
2 *ADT3* — white, coarse, stout grains.
3 *T3* — medium stature, oval, fine white grains, scented.
4 *T26* — white, coarse, stout grains.
5 Badsabhog — medium stature, oval, fine white grains, scented.

TABLE 2

% of loss of nutrients during washing (3 times with equal volume, W/V, of water) of rice.

Samples	Charnock	ADT3	T3	T26	Badsabhog	Mean
Total solid	0.65	0.50	0.54	0.32	0.64	0.53
Ash	9.8	11.4	4.7	7.6	6.5	8.0
Thiamine	4.9	3.9	5.5	3.7	5.0	4.6
Riboflavin	11.2	25.0	13.8	22.5	12.0	16.9
Niacin	13.4	10.1	6.9	6.6	8.6	9.1
Calcium	10.2	8.8	8.5	9.7	13.5	10.1
Phosphorus	5.7	6.6	2.3	3.0	5.8	4.7
Phytin-P	3.1	4.7	1.7	5.1	4.5	3.8
Iron	23.5	22.2	21.2	19.0	20.0	21.3
Available iron	24.5	34.5	26.5	20.6	17.6	24.7
Nitrogen	2.1	2.5	0.8	3.0	2.9	2.3

TABLE 3

Percent loss of nutrients in the gruel in rice cooked with 6 and 8 volumes of water.

Samples		Charnock	ADT3	T3	T26	Badsabhog	Mean
Total Solids	6V.[1]	9.3	6.7	6.8	6.3	8.4	7.5
	8V.[2]	9.7	8.3	7.8	7.9	10.8	8.9
Ash	6V.	19.1	15.5	16.1	25.6	18.7	19.0
	8V.	23.3	26.0	19.9	28.6	27.2	25.0
Thiamine	6V.	31.7	23.4	20.4	30.2	31.7	27.5
	8V.	41.7	31.1	30.6	31.3	38.4	34.6
Riboflavin	6V.	22.7	37.5	23.0	26.2	24.0	26.7
	8V.	27.0	46.7	28.4	40.6	28.8	34.3
Niacin	6V.	20.9	32.9	31.0	36.0	28.0	29.8
	8V.	35.0	36.3	34.4	46.2	44.4	39.3
Calcium	6V.	12.8	16.4	30.4	11.1	12.5	16.6
	8V.	19.3	24.7	36.6	25.0	17.5	24.4
Phosphorus	6V.	13.6	13.8	11.7	33.8	11.9	17.0
	8V.	15.2	16.6	14.1	35.8	16.0	19.6
Phytin-P	6V.	11.7	13.5	8.2	17.7	5.3	11.3
	8V.	17.4	16.7	9.7	38.8	14.9	17.5
Iron	6V.	24.4	22.2	24.0	8.1	10.1	17.7
	8V.	30.5	30.0	34.0	11.5	12.9	23.9
Available Iron	6V.	23.6	28.3	21.0	12.5	13.1	19.7
	8V.	30.9	31.6	24.0	18.4	16.9	24.3
Nitrogen	6V.	5.0	4.0	2.8	4.0	8.5	4.9
	8V.	6.8	7.4	4.0	9.2	13.7	8.2

[1] 6V. — Cooked with 6 volumes of water.
[2] 8V. — Cooked with 8 volumes of water.

Rice cooked with just sufficient water had no gruel to be drained off. Hence only the % retention of the nutrients, given in Table 4, was determined.

TABLE 4

Percent retention of nutrients in rice cooked with just sufficient water.

Samples	Charnock	ADT3	T3	T26	Badsabhog	Mean
Ash	90.4	88.5	94.7	92.5	92.4	91.7
Thiamine	88.2	93.6	90.9	95.9	98.1	93.3
Riboflavin	90.2	75.0	77.6	76.1	89.6	81.5
Niacin	86.7	89.5	92.6	92.5	98.7	92.0
Calcium	88.0	91.2	90.5	91.7	90.5	90.4
Phosphorus	94.0	93.3	97.9	96.8	92.7	94.9
Phytin Phosphorus	97.5	94.0	95.8	94.3	94.3	94.8
Iron	76.6	76.7	76.0	81.3	75.0	77.1
Available iron	74.5	64.5	72.5	79.4	81.3	74.4
Nitrogen	97.4	91.7	98.1	90.8	97.5	95.1

TABLE 5

Showing the digestibliity of rice cooked with different volumes of water. Amount of reducing sugar produced in mg.

Variety of rice	Rice cooked with water of					
	8 vols.	6 vols.	Just sufficient	8 vols.	6 vols.	Just sufficient
	With Takadiastase			With Pancreatin		
Charnock	9.0	7.7	7.1	5.3	4.4	4.0
ADT3	13.2	10.4	9.8	7.8	7.9	6.6
T3	7.6	7.5	6.3	4.6	4.5	3.5
T26	6.5	5.3	5.0	3.6	3.4	2.7
Badsabhog	9.8	9.5	7.9	6.3	6.1	5.3

DISCUSSION

The rice samples selected were raw rice obtained as paddy which were hulled to remove the husk only. There was no loss of the bran. The strains were known to have high thiamine values but it was found during this experiment that the ash, niacin and riboflavin contents of this strains were also fairly high. These values, given in Table 1, may not be comparable with those of Kik and Williams *(11)* probably due to the difference in the strains of rice used in this experiment.

It will be observed from the results given in Table 2 that the loss of a particular nutrient during washing of rice varies from strain to strain. The mean losses, however, are as follows: thiamine 4.6%, riboflavin 16.9%, niacin 9.1%, calcium 10.1%, phosphorus 4.7%, phytin phosphorus 3.8%, iron 21.3%, available iron 24.7% and nitrogen 2.3%.

The losses of thiamine, riboflavin and niacin during washing of these rice samples differ from those published by Kik and Williams *(11)*. These divergencies between the two results may be due to the difference in the strains used in these experiments.

It was found that cooking of different samples of rice (200 g.) with just sufficient water required 650 cc. to 700 cc. of water. The moisture content of each sample of rice when cooked with different volumes of water was found to be almost the same which is an indication that it was cooked approximately to the same degree. This was confirmed organoleptically and also by the fact that the variation in the weights of the cooked rice of different samples when cooked with different volumes of water was not marked.

The results in Table 3 show that, over and above the average losses during washing, about 28% of thiamine, 27% of riboflavin, 30% of niacin, 17% of calcium, 17% of phosphorus, 11% of phytin phosphorus, 18% of iron, 20% of available iron and about 5% of nitrogen are lost in the gruel when rice is cooked with 6 volumes of water and the gruel drained off. These results are in fair agreement with those of the retention of nutrients in the cooked rice. The losses of these nutrients in the gruel of rice cooked with 8 volumes of water were found to be higher and the retention of these nutrients in the cooked rice were found to be in fair accordance with the calculated figures. Kik and Williams *(11)* also used this volume of water for cooking. The average results in regard to the losses of thiamine, riboflavin and niacin, though they vary from strain to strain, are to some extent comparable with those given in Table 3.

Results in Table 4 show that the nutrients that remain after washing rice are not lost during cooking with just sufficient water. The values of these nutrients were in fair agreement with their values in washed rice (cf. Table 2).

The results in Table 5 show that the *in vitro* digestibility of the different rice samples are considerably different when cooked under the same condition. Basu and Sarkar *(2)* and Basu and Mukherjee *(3)* also observed that the *in vitro* digestibility of different rice samples (uncooked) were different. It will also be observed that the rice obtained after cooking with 8 volumes of water is most digestible *in vitro* and the rice obtained after cooking with just sufficient water is least digestible *in vitro*.

The fact would indicate that, although cooking with excess water, would entail losses in some nutrients, such cooking would probably be desirable where, as in some illnesses, digestion efficiency is poor.

SUMMARY

The average losses of nutrients during washing of five pure strains of rice prior to cooking have been found to be about thiamine 5%, riboflavin 17%, niacin 9%, calcium 10%, phosphorus 5%, phytin phosphorus 4%, iron 21%, available iron 25% and nitrogen 2.3%.

The average losses (over the loss during washing) of these nutrients in *gruel* of rice cooked with six volumes of water are thiamine 28%, riboflavin 27%, niacin 30%, calcium 17%, phosphorus 17%, phytin phosphorus 11%, iron 18%, available iron 20% and nitrogen 5%. These losses increase when the rice is cooked with 8 volumes of water and are 35%, 34%, 39%, 25%, 20%, 18%, 24%, 24%, and 8% respectively.

The retention of the nutrients in rice corresponds well with the values as calculated from loss of nutrients in the gruel. The loss of nutrients in rice cooked with just sufficient water is negligible.

It was also observed that the rice cooked with more water was more digestible *in vitro* than the rice cooked with less water. The *in vitro* digestibility of rice cooked with just sufficient water was the least.

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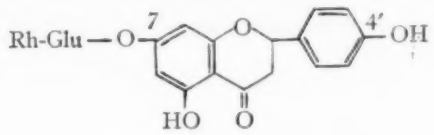
USE OF THE DAVIS METHOD TO ESTIMATE FLAVANONES

Naringin, the chief bitter principle of grapefruit, is a flavanone glycoside having the structure I (Rh = rhamnose; Glu = glucose). Recently, there has been much interest in the possibility of debittering grapefruit juice enzymatically *(3, 4, 5, 6)*. In this procedure, the juice is treated with an enzyme ("naringinase") which hydrolyzes various glycosides, including naringin. There is evidence that hydrolysis of the naringin proceeds in stages to



(I)

give prunin (naringenin glucoside) and rhamnose, followed by hydrolysis of the prunin to give naringenin and glucose. Naringenin is not bitter while prunin, although perceptibly bitter, is not nearly as bitter as naringin[a]. It is convenient to follow the progress of the debittering by means of the Davis color method *(1)* for the determination of flavanones. In practice, however, it has been found that considerable debittering occurs before there is any appreciable disappearance of naringin, as indicated by the Davis method *(3, 5)*. This has been explained *(3)* by assuming that both the bitter naringin and the relatively non-bitter prunin develop about the same amount of color in the Davis method, while the non-bitter naringenin, which is formed in the last stage of the hydrolysis, develops less color than either. It is the purpose of this article to point out the proper use and limitations of the Davis method.

In the procedure described by Davis, the sample is dissolved in diethylene glycol and made about 0.08 *N* in sodium hydroxide. After 5 minutes the yellow color is measured in a colorimeter, using a blue filter which transmits in the approximate range 400-500 mμ. Reference to Figure 1 shows that, under the specified conditions of alkalinity, both naringin and prunin have a very strong absorption band at 427 mμ, while naringenin has no significant absorption in this region. It is futile, therefore, to attempt to estimate naringenin by absorption measurements in the 400-500 mμ region, particularly since there are other substances in citrus which may interfere by virtue of their own strong absorption in this range in alkaline solution. This interference will probably not have a serious effect on the estimation (in grapefruit) of the very preponderant naringin. However, difficulties have been encountered in trying to correlate naringin values as determined by the Davis method with grapefruit bitterness during a given season or from one season to another. This may conceivably be due to a natural hydrolysis in the fruit of naringin to prunin. Hall *(4)* has already suggested that a hydrolytic process may occur during ripening and he was able to prepare

[a] At a concentration of 5×10^{-4} *M*, prunin was somewhat bitter and naringin intensely so. At 5×10^{-5} *M* prunin was almost tasteless, while naringin remained quite bitter. The sample of prunin had been prepared by partial hydrolysis of naringin (2) but appeared to be essentially free of the latter compound.

an enzyme from grapefruit leaves and peel which catalyzed the hydrolysis of naringin to naringenin *in vitro*. Whether prunin and naringenin do occur in the ripe fruit is a question which remains to be answered, since almost nothing is known about the flavonoid composition of grapefruit.

Hesperidin, the most abundant flavonoid in oranges and lemons, is frequently estimated by application of the Davis method. Reference to Figure 1 reveals that this compound, like naringenin, absorbs only feebly at wavelengths greater than 400 mμ. Therefore, attempts to estimate hesperidin by the method in question cannot be expected to yield meaningful results. In fact, it would probably be better to estimate hesperidin and the accompanying flavanones simply by measuring the height of the absorption band which occurs at 285 mμ in neutral solution.

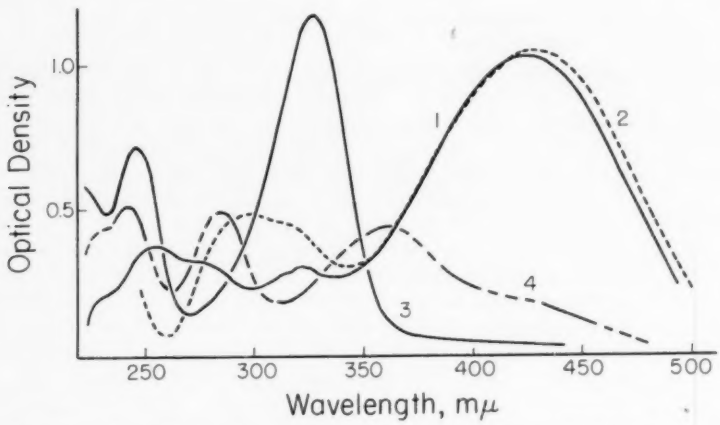
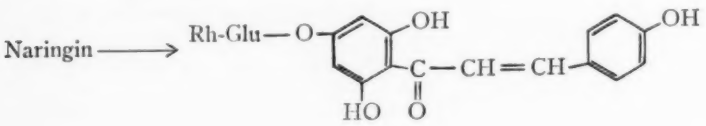


Figure 1. Absorption spectra in 90% diethylene glycol–0.08 *N* NaOH; flavanone concentration: 3.8×10^{-5} *M* (1) Naringin; (2) Prunin; (3) Naringenin; (4) Hesperidin. (4) Hesperidin.

The marked difference between the absorption curves of naringin and prunin compared with those of the other flavanones appears to reside in the fact that naringin and prunin are very readily transformed to their chalcones in weakly alkaline solution:



The band at 427 mμ is certainly due to the ionized chalcone. This facile conversion to the chalcone is probably characteristic of flavanones having both a protected 7-hydroxyl and a free 4′-hydroxyl group. Other flavanones

either do not chalconize readily or, if chalconized, do not absorb strongly (in the ionized form) beyond 400 mμ.

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